המועצה המדעית לישראל

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IN HONOUR
OF
PROF. ANDOR FODOR'S
70TH BIRTHDAY

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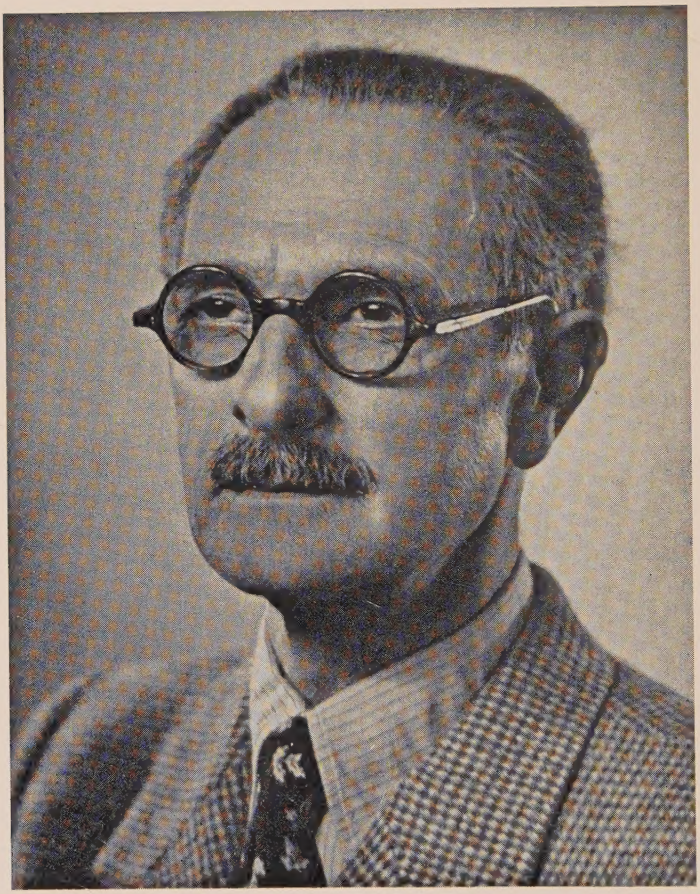

# PROF. ANDOR FODOR

Rarely today has a scientist been privileged not only to take an active part in the development of a new branch of science, but to become its initiator and first teacher in a new nation. Professor Andor Fodor, to whom this issue is dedicated in honour of his 70th birthday, has achieved such a distinction.

Born in 1884 into the wealthy Jewish Hungarian community of Budapest, Fodor chose science as his calling and chemistry as his profession at a time when classical organic chemistry developed rapidly under outstanding teachers in the famed schools of Central Europe. Fodor studied chemistry in Zurich at the Federal Polytechnical College from 1903 to 1909 with F. P. Treadwell, Eugen Bamberger and E. Berl. He received his Ph.D. for a thesis on the alkaline saponification of cellulose and glucose nitrates. Then, in spite of the fact that biochemistry was looked upon as a somewhat speculative and therefore a little suspicious subject scientifically, he decided to continue his work at von Euler's Institute for Physiological Chemistry at Stockholm, one of the few European institutes dedicated to biochemical research. As Fodor once explained to this writer, in choosing biochemistry he was influenced (apart from purely scientific reasons) by the wish to work in a branch of chemistry pursuing only peaceful aims.

In 1912 Fodor joined Abderhalden's Institute in Halle, Germany, where the classical organic chemistry of Fischer was combined with the modern biological-dynamic and physicochemical trend. He remained at Halle for the next 12 years, until 1923, when he emigrated to Israel.

Fodor's years of intense scientific activity at Halle gave him the fundamental concepts on which he based his later research work on enzyme and protein structures and enzyme activity. He studied the mathematical principles underlying colloid and enzyme chemistry and wrote a comprehensive treatise on the mathematical treatment of biological problems, which forms part of Abderhalden's famous *Handbuch der chemischen Arbeitsmethoden*. But concentrating mainly on original research, he undertook a number of fundamental researches on the properties and behaviour of colloids since he saw in the correct interpretation of colloidal phenomena an essential and valuable tool for the understanding of biochemical reactions. Researches on adsorption, especially that of amino acids, peptides and proteins, interested him most, and he did much for the systematization and clarification of fundamental concepts in colloid chemistry. The term "lyosorption", denoting the adsorption by solvated colloids by means of their solvent layer, was introduced by him into literature.

In 1910 Fodor married and a son was born to him in 1912. He had entered Abderhalden's Institute in 1912 as a senior assistant; in 1919 he became "Privatdozent" (lecturer) and three years later Professor. After this nomination — quite an achieve-

ment for a Jewish non-German citizen — Fodor seemed assured of a life of unintei rupted scientific activity. But he had long been an active member of the Zionist orga nization in Germany and was known in Jewish circles as an ardent follower of th Zionist movement. One of the few German Jews to realize their ideals at the time, h emigrated to Palestine in 1923, following the invitation of Chaim Weizmann to establis an institute for chemical research and teaching at the newly founded Hebrew Univei sity of Jerusalem.

The Palestine of 1923 was a typical backward Middle East country. In Jerusalem even the most basic necessities for laboratory research — electricity, water, gas – were non-existent. The university, located on Mount Scopus and reachable from th city by horse and carriage or by a half hour's walk under the scorching Mediterranea sun, literally had to be built from scratch: an abandoned Arab house served as th chemical laboratory, research was carried out under the most primitive conditions isolated from European and American research centres. It testifies to Fodor's spiri that even during the most difficult periods he never interrupted research and consistentl continued to publish papers.

In 1925 Fodor was made professor ordinarius and Director of the Department o Biological and Colloidal Chemistry in the Faculty of Mathematics and Natural Sciences in 1931 he became Head of the Division of Biological Studies. Always highly esteeme by his co-workers and students, many of whom were enabled by his help to establis their own branches of research at the university, he was twice elected Dean of th Faculty of Mathematics and Natural Science.

The years 1947-8 brought the establishment of the State of Israel and our War o Liberation, but were sorrowful ones for Professor Fodor. The University had to abando its home on Mount Scopus, to which, in defiance of the conditions stipulated in th Armistice treaty between Israel and Transjordan, we are denied access.

After some years of uncertainty when the Department of Biochemistry was house in Jerusalem under almost as primitive conditions as in its beginning, Professor Fodo — prior to his retirement in 1953 — could see the construction of new better equippe laboratories and witness the development of the faculty which he had started fror small beginnings to a modern centre of research and teaching activity.

Today a large part of biochemical research is based on the fundamental concept formed during the same years that Prof. Fodor carried out his main scientific investi gations. Chemists were then concerned with the protein nature of enzymes, their de finition as organic substances, the mode of combination between enzyme and its sub strate and the reaction kinetics of enzymes. They were all experimentally treated i Fodor's numerous papers on the yeast peptidases, which he chose to study since th hydrolytic enzymes were the easiest to isolate and lent themselves to both experimenta and theoretical treatment with relatively simple means. As a preliminary step, h synthesized a number of peptides in a search for proteinases and peptidases. The syn thesis by Fodor together with Abderhalden of a peptide containing 18 leucine an glycine residues was an outstanding achievement. Contrasting with Willstaetter's con tention that he had isolated a protein-free enzyme (*invertin*) Fodor, from the beginning stressed the importance of the colloidal nature of the enzymes for their reactivity, theory which led almost automatically to the conclusion that the enzymes must b

proteins. Accordingly, he treated kinetic questions by assuming the enzyme-substrate complex to be an adsorbate and by regarding the Michaelis-Menten concept as a special case rather than as the rule.

Substrate specificity questions led Fodor to the formulation of his *Traegertheorie*. He had extracted from yeast cells two aminopeptidases, one acting preferably on dipeptides, the other on tripeptides. The tripeptide splitting activity could be separated by adsorbing the enzyme extract on kaolin and eluting the adsorbate with glycine solution. Enzyme solutions almost completely devoid of protein could be obtained by this method. It should be remembered that in 1952 Binkley obtained very similar results when, he purified the enzyme, cysteinylglycinase. The dipeptide splitting activity could be restored to the eluate by adding to it an ultrafiltrate of the original yeast extract containing a slowly dialyzing co-factor which was not a metal ion. Concurrently the substrate specificity of the enzymes was not constant but varied in regard to leucyl and glycylpeptides, according to the free aminoacids or products of protein hydrolysis added to the solutions, notably leucine and glycine. Contrary to Grassman's contention that every amino acid in a peptide split by aminopeptides had to have its own distinct enzyme, Fodor postulated the presence in the cell of a carrier body which interacted with a number of different cofactors, called by him "zymoactive" substances, thus producing peptidases of different specificity according to the carrier and cofactor involved.

The clear formulation of the apoenzyme-coenzyme correlation in the twenties, when no coenzyme had been isolated and enzyme chemistry more often than not took the shape of mere speculation, was a feat of remarkable intuitive insight, the more so as the whole research was undertaken with an enzyme whose properties are still controversial. The same peptidase Fodor found was isolated over 15 years later by Smith, who essentially confirmed Fodor's earlier results. Although Smith's enzyme was electrophoretically uniform, its protein could be partially denaturated without destroying the activity, showing that it was still impure.

Fodor's second great subject of research was protein structure. His method for the degradation of proteins was to heat them under anhydrous conditions with such agents as glycerol, acetic anhydride, resorcinol or beta-naphthol. He obtained low molecular, water soluble compounds which he termed "acropeptides" and found them to contain closed peptide chains. Furthermore, he claimed that these closed units were repeating units, meaning that the arrangement of the amino acids in a protein was not entirely random, but that the polypeptide chain consisted of a number of recurring units, each consisting of 4 to 8 amino acid residues. The theory met with opposition mainly because the closing of the peptide chains was explained by assuming the formation of covalent bonds. (The concept of hydrogen bonds was introduced only much later.)

The presence in nature of closed peptides, like the hormone oxytocin and vasopressin, both closed hexapeptides with a dipeptide side chain, and proteins with no free amino or carboxylic end groups has since been amply demonstrated. It should be recalled that the 6-2 structure found for the hormones mentioned was found by Fodor when he cleaved gelatin with hot anhydrous glycerin.

As a scientist living in a young and progressive country, Fodor was well aware of the help which scientific research could provide in its upbuilding. He twice made agricultural problems the subject of his researches. In 1925 he published a comprehensive

paper on tobacco fermentation (with the late A. Reifenberg) which laid the ground for improving methods of treatment of locally grown tobacco leaves. In 1939 he found that orange peels contained much sugar and developed a method for extracting it.

In conclusion, we express to Professor Fodor our best wishes for many long years of well-earned rest to cultivate the numerous cultural interests which his strenuous scientific work did not always allow him to pursue fully.

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*Jerusalem*

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# STUDIES ON THE ACTIVITY AND INHIBITION OF YEAST ESTERASE

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Although the lipolytic activity of yeast cells was detected more than 40 years ago[1] there are very few references on this subject in the literature. In the only detailed report available[2] no attempt was made to elucidate the kinetics of the enzymatic action nor the substrate specificity of the latter. Moreover, it was evident from preliminary experiments carried out in Israel with various yeasts supplied by local manufacturers that the nature of the lipases may vary remarkably between one yeast and another as to their activity and their optimal pH requirements.

This study was mainly carried out with commercial bakers' yeast (*Saccharomyces Cerevisiae* Hansen) manufactured by the Fleischmann Laboratories, Bronx, New York. Samples of beer yeasts (bottom yeasts) supplied by the courtesy of the Ruppert Brewery, New York, and the Wallerstein Laboratories, New York, did not show any lipolytic activity under the experimental conditions. It has already been observed by Gorbach and Guentner[2] that the lipolytic activity of yeasts is dependent upon the conditions of growth, although generally beer yeasts are poorer in lipase than bakers' yeasts*. Whereas olive oil was the only substrate employed in their report, the yeast used in the present investigation was unable to hydrolyze olive oil at all, which, moreover, inhibits strongly the hydrolysis of ester substrates. Thus, the rather narrow range of substrate specificity observed with our yeast enabled us to investigate the influence of enzymatically resistant esters on the lipolytic activity in vitro.

## EXPERIMENTAL

### *The substrates*

The following esters were either prepared in the laboratory or purchased commercially and then purified according to standard procedures: Ethylacetate, ethylpropionate, methylbutyrate, ethylbutyrate, *n*-butyl-*n*-butyrate, ethyl-*n*-caproate, methyl-*n*-laurate, *n*-butyl-acetate, triacetin, tripropionin, tributyrin, triheptylin, tricaproin and olive oil.

### *Buffer mixtures*

For the pH curve the m/15 phosphate mixture was employed in the range between pH 5—7.5. In the lower pH range mixtures of $KH_2PO_4$ and HCl were used and below pH 2, 0.02N $H_3PO_4$ was employed. The pH above 7.5 was adjusted by M/15 mixtures of $(NH_4)_3PO_4$ and $NH_3$. Thus the basic composition of the buffers in the whole range

* The relation between the composition of the culture medium and the lipase content of aspergillus and of penicillium has been the subject of recent studies[3,4].

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between pH 1.5 to 11 remained unchanged. In addition, McIlwain's buffer mixture and borate buffer in the acid range were used.

*The enzymes*

The yeasts were obtained from the manufacturers as fresh as possible. Pressed yeasts were kept at 4°C, carefully wrapped in wax paper to prevent drying, while non-pressed yeasts were stored in tightly closed jars. After five days' storage the yeast was rejected and replaced with fresh samples. For the enzyme experiments they were blended with three times their weight of distilled water, washed and centrifuged four times and brought up with distilled water to their former volume. Observing these precautions constant results were obtained at the alkaline pH optimum (pH 7.5) whereas at the acid peak (pH 2.8) some variations of the kinetics of the enzymatic action were observed, although here, too, the maximal per cent hydrolysis remained unchanged.

*Method of assay*

Since formaldehyde is rapidly oxidised by yeast the formol titration used in earlier investigations was replaced by titration of acidity in alcohol with 0.05 N or 0.025 N alcoholic KOH from a microburette using phenolphthalein as indicator. For the enzyme experiments two millimoles of the monohydric alcohol esters or 2/3 millimoles of the triglycerides were dissolved or emulsified in 20 ml of buffer mixture to which 6 ml of the yeast homogenate (1 part of yeast to 3 parts of distilled water) were then added. Ethylbutyrate and ethylacetate were used in a concentration of 1.73 millimoles in 20 ml. Aliquots of 4 or 5 ml, as indicated, were removed for titration. The incubation temperature was 30°C. All experiments were performed in quadruplicate.

## DISCUSSION OF THE RESULTS*

This yeast esterase has a rather narrow range of activity with respect to both the monohydric alcohol and the glycerol ester homologue series employed. In the former series the isomers ethylbutyrate and butylacetate are hydrolyzed with the same initial velocity, but the maximal per cent hydrolysis (after 90 minutes) is 27 per cent for ethylbutyrate against 21 per cent for butylacetate. Ethylcaproate likewise is hydrolyzed with the same initial velocity which, however, falls off sharply after the first 20 minutes of incubation and reaches a maximum hydrolysis of only 13 per cent after 90 minutes. All the other substrates of higher and lower molecular weight used, including the triglycerides, are hydrolyzed with lower initial velocities or are not hydrolyzed at all (Table I).

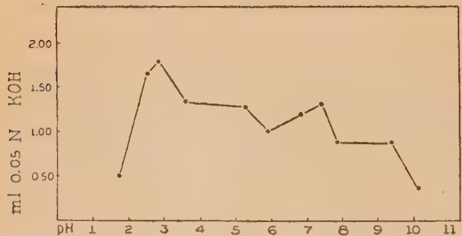


Figure 1

*Dependence of ester hydrolysis by yeast homogenates upon pH of buffer.* Substrate: Ethylbutyrate. Concentration of enzyme 6.45 mg of nitrogen per ml of homogenate. Time of incubation 60 minutes. 5 ml aliquots were removed for titration out of a total of 26 ml enzyme-substrate-buffer mixture. All values are corrected by the blanks.

* Since both brewery yeasts employed showed no lipolytic activity this discussion refers to bakers' yeast only.

TABLE I

*Activity of yeast homogenate toward various esters.* Concentration of the esters as indicated in experimental part. Concentration of the enzyme corresponds to 6.45 mgN per ml of homogenate. Buffer: Phosphate pH 7.5. Temperature of incubation 30°C. Values were estimated from the linear parts of the hydrolytic curves and are expressed as micromoles of KOH consumed per hr. per mg of enzyme nitrogen.

| *Substrate* | *Activity* |
|---|---|
| Ethylacetate | 0 |
| Ethylpropionate | 0 |
| Ethylbutyrate | 17 |
| Ethylcaproate | 17 |
| Butylacetate | 17 |
| Methylbutyrate | 7 |
| *n*-butyl-*n*-butyrate | 6 |
| Methyllaurate | 0 |
| Triacetin | 5 |
| Tributyrin | 13 |
| Tripropionin | 6.5 |
| Trivalerin | 2 |
| Tricaproin | 0 |
| Triheptylin | 0 |
| Olive oil | 0 |

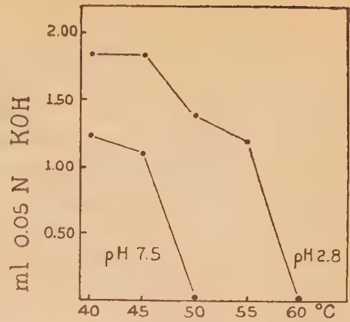


Figure 2

*Heat inactivation of yeast homogenates at various temperatures.* Yeast homogenates of the same concentration as in Figure 1 were heated for thirty minutes to the temperatures indicated on the abscissa, and then immediately cooled. Substrate: Ethylbutyrate. Time of incubation: 60 minutes. Phosphate buffer of pH 2.8 and 7.5. 5 ml aliquots were removed for titration out of 26 ml enzyme-substrate-buffer mixture. All values corrected by the blanks.

As shown in Figure 1 there are two optima for hydrolysis, one in the alkaline range at pH 7.4 and one on the acid side at pH 2.8. This is at variance with the report cited previously[2] where both for brewers' and bakers' yeast an optional range between pH 6.6—6.8 was found. Using ethylbutyrate and phosphate buffer the hydrolysis at pH 7.4 proceeds linearly in the first 20 minutes' interval, which is true also with reference to all the other esters used. In the interval of 7.8 mg—3.22 mg enzyme nitrogen per ml of homogenate the rate of hydrolysis was found to be proportional to the concentration of the enzyme. At the acid optimum, autolysis of the yeast is fairly strong; here two different curves of hydrolysis were observed. In one case hydrolysis proceeds with an initial velocity which is nearly twice as high as that observed at the alkaline optimum; in the other instance, the curve is S-shaped and hydrolysis is very slow in the first twenty minutes' interval of incubation and then rises steeply. In both instances, however, maximum hydrolysis is achieved by the enzyme after 90 minutes and corresponds to about 30 per cent. The cause for the difference in the kinetics at this pH could not be ascertained but it is independent of the experimental conditions. Hence, only the initial activities toward the esters at pH 7.4 have been tabulated, which were observed to be constant.* As shown in Figure 2, hydrolysis at this pH is completely destroyed by pretreatment of the yeast homogenate at 50°C for thirty minutes, while hydrolysis at pH 2.8 still retains a considerable margin of activity which is destroyed only by heating at 60°C. Lipolysis by this yeast seems thus to be concerned with two enzyme proteins, differing in their heat stability, as well as in their optimal pH requirements. In general, however, yeast esterase was found to be extremely labile and easily destroyed by autolysis or plasmolysis. Plasmolysis performed by freezing samples of pressed

* Substantial fluctuations in the initial rates of the hydrolysis of olive oil by commercial bakers yeast under constant experimental conditions were reported by Gorbach and Guentner.

yeast at – 80°C or by grinding them with dry ice for thirty minutes resulted in a complete loss of their activity, both at the acid and the alkaline pH optima. Likewise, treatment of pressed yeast with ethylacetate, followed by precipitation of the plasmolysate with acetone at – 15°C resulted in a complete inactivation. (In both instances sucrose and glucose were still strongly fermented). Incubation at 37°C of yeast suspensions in phosphate buffer or in distilled water did not change their activity unless continued for three hours, when it resulted in an activity decrease. If the yeast suspensions were incubated for one hour and then centrifuged at 5000 r.p.m. no activity was found in the supernatant, whereas the pellet, if brought up with water to its former volume, showed the same activity as the non-centrifuged homogenate. Under the experimental conditions, therefore, the esterases are liberated by the cells only upon the stimuli of the substrates and are not stable once the cell structure has been destroyed. Free butyric acid is not absorbed by the yeast cells under the experimental conditions, as could be ascertained by the constancy of both pH and titrable acidity in cell suspensions to which butyric acid was added up to a concentration of 0.5 millimole per 10 ml; thus the titrated increase in acidity in the enzyme experiments is certain to correspond to the total of the acid liberated by enzymatic action. The highest activity was found in the presence of phosphate buffer, or, like McIlwain's buffer mixture, in buffers consisting in large part of phosphate. No difference in enzymatic activity was thus found at pH 7.5, employing either phosphate or McIlwain's phosphate-citrate mixture*. On the other hand, in citrate buffer of pH 2.8 enzymatic activity is very low. In phthalate buffer of the same pH there is no ester hydrolysis. One part of the phthalate buffer added to three parts of a $KH_2PO_4$ — $H_3PO_4$ mixture at pH 2.8 failed to exert any inhibitory effects. The presence of phosphate ions thus seems to be the essential for ester hydrolysis by this yeast. The narrow range of the substrate specificity of this esterase furnished a good opportunity for studying the influence on the enzymatic hydrolysis of esters related to the substrates used but which by themselves are not attacked by the enzyme. Thus, the influence of varying amounts of ethylacetate, methyllaurate, ethylpropionate, tricaproin, triheptylin and olive oil on the hydrolysis of ethylbutyrate and tributyrin has been investigated, as well as that of sodiumcholeate, the inhibitory effect of which on pancreas lipase under certain conditions has been shown in other studies[5]. Figure 3 demonstrates a relationship between inhibitor concentration and inhibitory effect very similar to the one aforementioned. Linear functions are obtained if the inhibitor concentration on the abscissa is given as its natural logarithm in the interval between 0.03 to 0.30 ml per 26 ml of enzyme-substrate-buffer mixture. While none of the inhibitors reduces enzymatic hydrolysis to zero, water soluble esters such as ethylacetate and ethylpropionate do not cause any inhibitory effect, because the less soluble ethylbutyrate is absorbed more readily by the enzyme protein, or by the surface of the cells. On the other hand, insoluble esters like tricaproin, methyllaurate or olive oil are absorbed in preference to their more soluble homologues and, since they are not hydrolyzed, "block" the access to the enzyme protein surface for the more soluble esters which on their part, in the absence of the inhibitor, are attacked by the yeast esterase. Taking the inhibitory effect of an ester as the measure of its adsorption on the enzyme protein surface the inhibition

* Borate buffer at pH 7.4 causes a heavy sedimentation of the yeast cells and therefore cannot be employed.

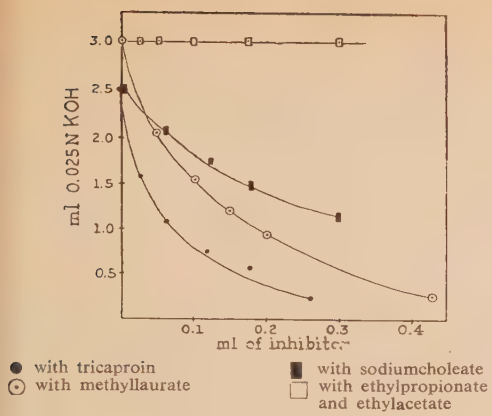


Figure 3

*Inhibition of the ethylbutyrate hydrolysis in the presence of varying amounts of inhibitors.*

The numbers on the abscissa indicate ml of inhibitor in 26 ml of enzyme-substrate-buffer mixture. With respect to sodiumcholeate this refers to a 10 per cent solution. Time of incubation: with methyllaurate 90 minutes, with all other inhibitors 60 minutes. Enzyme concentration: 6.45 mg nitrogen per ml of homogenate. 5 ml of the total mixture were removed for titration.*

* In the presence of olive oil and triheptylin similar curves to that with methyllaurate and tricaproin were obtained but have been omitted for the sake of greater clarity.

of the hydrolysis of the soluble esters in the presence of less soluble ones can be explained by the laws governing the adsorption on colloidal surfaces of insoluble compounds in the presence of their soluble homologues**.

Sodiumcholeate, characterized by its high surface activity, occupies an exceptional position by being water soluble and not related in chemical nature to the substrates used. Its role either as an inhibitor or an activator of ester hydrolysis by adsorption on the substrate water interface has been discussed previously[5].

** A similar inhibition phenomenon has been reported concerning the hydrolysis of short chain cholesterol esters by pancreatic enzymes in the presence of their higher homologues[6].

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# PEPTIDASES IN RENAL AND HEPATIC TISSUES*

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## INTRODUCTION

Table I summarizes earlier observations in this Laboratory on the distribution of the peptidases in kidney homogenates which are effective on chloroacetyl and glycyl-*L*-, *D*-, and dehydroamino acids[1-9]. It will be noted that (a) the greater part of the activity of the homogenate toward the chloroacetylamino acids and toward the glycyl-*L*-amino acids is soluble, whereas (b) the greater part of the activity toward the glycyl-*D*-amino acids and toward the glycyldehydroamino acids is associated with the particulate fraction.

TABLE I

*Approximate distribution of peptidases in kidney homogenates toward chloroacetyl- and glycylamino acids*

| *Substrate* | *Per cent of homogenate in* | | | |
|---|---|---|---|---|
| | *Supernatant* | *Designation of enzymes* | *Sediment* | *Designation of enzymes* |
| Chloroacetyl-*L*-amino acids | 100 | Acylases I and II[1] | 0 | — |
| Chloroacetyl-*D*-amino acids | 0[2] | — | 0 | — |
| Chloroacetyldehydroalanine | 100 | Acylase I[3] | 0 | — |
| Chloroacetyldehydroamino acids other than dehydroalanine | 0 | — | 0 | — |
| Glycyl-*L*-amino acids | 90 | *L*-Dipeptidase and Acylase I[6] | 10 | Aminopeptidase[4] |
| Glycyl-*D*-amino acids | 10 | ?[7] | 90 | Aminopeptidase[4] |
| Glycyldehydroalanine | 20 | Acylase I | 80 | Aminopeptidase[4, 5] |
| Glycyldehydroamino acids other than dehydroalanine | 10 | ?[8] | 90 | Aminopeptidase[4] |

1. Acylase I acts on all acylated-*L*-amino acids except acylated-*L*-aspartic acid which requires acylase II.
2. Chloroacetyl-*D*-methionine is very slightly susceptible to acylase I, about one ten thousandth as much as the corresponding chloroacetyl-*L*-methionine.
3. Earlier referred to as dehydropeptidase II.
4. This enzyme acts upon glycyl-*L*-, *D*- and dehydroamino acids at a nearly equal rate.
5. Earlier referred to as dehydropeptidase I.
6. The greater part of the activity is due to that of *L*-dipeptidase. It can be distinguished from that due to acylase I by the extreme lability of the *L*-dipeptidase activity.
7. The activity of the soluble fraction toward glycyl-*D*-amino acids is not associated with acylase I or any enzyme entity so far characterized.
8. Traces of this soluble activity are associated with acylase I, but the greater part is unknown.

It has been impossible so far to disassociate the activities (a) of acylase I toward chloroacetyldehydroalanine and the chloroacetyl-*L*-amino acids, (b) of acylase I toward chloroacetyl-*L*-amino acids, glycyl-*L*-amino acids and glycyldehydroalanine, and (c)

* Dedicated to Professor A. Fodor on the occasion of his 70th birthday.

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of aminopeptidase toward glycyl-*L*-, *D*- and dehydroamino acids. It would appear that the soluble renal acylase I possesses a high order of optical specificity, acts more rapidly on chloroacetyl- than on corresponding glycylamino acids, and is restricted in its activity among the dehydropeptides toward the derivatives of dehydroalanine*. On the other hand, the particulate renal aminopeptidase is indifferent toward the configuration of the terminal amino acid residue, and requires only that the acyl amino acid residue carry a free α-amino group**.

These results on kidney tissue are, with a single exception, reasonably clear-cut and understandable. The exception concerns the observation that although acylase I acts readily on chloroacetyl- and glycyl-*L*-amino acids, it acts only on chloroacetyl- and glycyldehydroalanine of all the dehydropeptides tested***. This is rather puzzling, unless it is assumed that there is something unique about the dehydroalanyl configuration which sets it apart from that of other dehydroamino acids, and thereby renders it susceptible to the action of the enzyme. No evidence for such an assumption is, however, available. It was therefore considered desirable to attempt some illumination of this problem by studying the distribution of similar peptidase activity in other tissues, chiefly in liver and in hepatoma. In view of earlier data on these tissues[1-6], it was realized that a somewhat different picture of the distribution of the peptidase activities would emerge.

Thus, a comparison of the susceptibility of glycyldehydroalanine and of glycyldehydrophenylalanine in liver and in hepatoma revealed a marked rise in that of the former substrate as a result of the neoplastic transformation, and a practical disappearance of that of the latter substrate[3]. A similar situation to that of glycyldehydrophenylalanine occurred in the case of chloroacetyldehydroalanine[5]. Yet the activity toward chloroacetyl-*L*-amino acids, including that of chloroacetyl-*L*-alanine, was very nearly the same in liver and in hepatoma[5]. In the course of another comparative study[6], although the activity toward glycyldehydroalanine was increased in the hepatoma as compared with that in the liver, the activity toward glycyldehydronorvaline was changed little if at all. These observations are to be considered against the findings that in a wide variety of tissues studied, the greater part of the activity toward glycyl-*L*-alanine was always soluble, that toward glycyl-*D*-alanine was always particulate, and

* In some features, as in its specificity toward peptide bonds involving *L*-amino acids, its need for a free terminal α-carboxyl group, and in its diminished activity toward substrates possessing a free α-amino acyl group, renal acylase I resembles pancreatic carboxypeptidase: it differs from the latter in its high order of activity toward aliphatic-substituted terminal amino acid residues, and in its activity toward peptide bonds involving a terminal dehydroamino acid residue.

Renal acylase I probably traces its ancestry back to Schmiedeberg histozyme. Its high order of optical specificity has permitted its use in the enzymatic resolution of α-amino acids. Renal acylases other than I and II apparently occur in the kidney, for there is some evidence[7] that a separate acylase, resembling pancreatic carboxypeptidase, is present and acts preferentially on acylated aromatic-substituted amino acids.

Cobalt augments acylase I activity toward the less susceptible substrates and inhibits the activity toward the more susceptible substrates[7]. In either case, the effect on corresponding chloroacetyl- and glycylamino acids is the same.

** Aminopeptidase activity is affected by the optical configuration of the acyl amino acid residue, but to no such extent as is acylase I. The former will attack *D*-peptides at a slower rate than *L*-peptides, but the latter enzyme is almost inert toward any substrate containing a *D*-amino acid residue in any position. Neither acylase I nor aminopeptidase has more than a trace of activity toward *L*-amino acid amides.

*** These included the derivatives of dehydro-alanine, butyrine, valine, norvaline, leucine, and isoleucine, as well as the corresponding saturated derivatives.

that toward glycyldehydroalanine, with the exception of the kidney, was always soluble[10]. It might therefore have been anticipated that in the distribution of the peptidase activities involving dehydroalanyl substrates the kidney might be atypical, or the susceptibility to dehydroalanyl substrates might be atypical.

The present studies have been concerned with rat and mouse liver, and with a transplanted mouse hepatoma. The first observations were made on the differential heat denaturation of the soluble fraction of rat liver homogenate in respect to the three substrates, chloroacetyl-*L*-alanine, chloroacetyldehydroalanine and glycyldehydroalanine. In the comparable soluble fraction of kidney, the hydrolysis of these three compounds is attributed to a single enzyme entity, namely acylase I. All methods of fractionation used, including that of heat denaturation, failed to indicate any separation of the activity in kidney toward the three substrates. In liver, however, the activity toward the three substrates was readily separable. Thus, at 48° the activity of the soluble liver fraction toward glycyldehydroalanine decreased about 90%, whereas that toward the other two substrates was nearly unaffected; at 60°, the activity toward chloroacetyl-*L*-alanine decreased about 90%, whereas that toward chloroacetyldehydroalanine was nearly unaffected. In the fresh soluble liver fraction, the ratio of activities toward chloroacetyl-*L*-alanine and chloroacetyldehydroalanine was about 10:1, and after the heating procedure the ratio was about 1:1*. This relatively heat-stable and soluble fraction of liver, earlier referred to as dehydropeptidase II[5], is the activity which apparently disappears when the liver becomes neoplastic. The second soluble liver fraction, namely that toward chloroacetyl-*L*-alanine, apparently remains at the same level when the liver becomes neoplastic, while the third soluble fraction, namely that toward glycyldehydroalanine, increases as a result of the neoplastic transformation. The present experimental observations are gratifying insofar as they clarify some of the earlier observations by Greenstein, Fodor and Leuthard[5] on the changes in levels of certain hepatic peptidases as a result of the cancerous alteration of the tissue. As far as comparison with the corresponding renal system is concerned, it is evident as expected that the distributions of enzymes in one tissue, together with their individual specificities, are not necessarily the same as in another tissue. There is nothing in hepatic tissue, whether normal or neoplastic, which resembles renal acylase I, unless it is the heat-stable dehydropeptidase II fraction in normal liver. But even here, the nearly equal activity toward chloroacetyl-*L*-alanine and chloroacetyldehydroalanine, compared with the corresponding ratio of 15:1 for renal acylase I, suggests that further purification might remove the activity toward the saturated peptide from the hepatic dehydropeptidase II preparation and yield the long-sought goal of clear-cut evidence for a specific dehydropeptidase. Work on this problem is in progress.

The activity of the mouse liver homogenate toward glycyldehydronorvaline is divided between the soluble and particulate fractions, whereas that toward glycyldehydrophenylalanine is entirely in the particulate fraction. As shown by others[11], the neoplastic transformation of liver is attended by a considerable depletion in the particulate systems. Thus, it is no surprise that the activity in the hepatoma toward glycyldehydrophenylalanine practically vanishes. In the case of glycyldehydronorvaline, however, although the activity toward this substrate in the hepatoma particulates also decreases, in the

* The corresponding ratio for renal acylase I is about 15[9].

supernatant it markedly increases, so that the specific activities in homogenates of liver and hepatoma are nearly equal. Most of the activity of liver and hepatoma homogenates toward glycyldehydroalanine is soluble, but here too a decrease in particulate activity in the hepatoma occurs, together with a marked increase in activity of the tumor supernatant.

It may be advisable at this stage to emphasize the fact that such difficulties in transposition of interpretations of peptidase distribution from one tissue to the other occur only when dehydropeptide substrates are considered. Thus, with a large variety of chloroacetyl- and glycyl-*L*-amino acids studied under identical conditions with homogenates of kidney and liver, the ratios of hydrolytic rates for each substrate by the two tissues were in every instance close to 3. With a similar group of glycyldehydroamino acids the corresponding ratios varied from 32 for glycyldehydroalanine to 155 for glycyldehydronorvaline and to 650 for glycyldehydrophenylalanine[9]; the ratio for chloroacetyldehydroalanine was 7. Again, it will be recalled that the *L*-peptidases are chiefly in the soluble fraction of all tissues studied, the *D*-peptidases are in the particulate fraction, while the glycyldehydroalanine activity is soluble in all tissues except for kidney. There is usually something exceptional in the susceptibility behaviour and distribution of the dehydropeptidases, and it is the curious position of this class of enzymes which is so agreeably challenging. A table of distribution in hepatic tissues, similar to that of Table I for kidney, will be assembled after further information on a greater variety of substrates has been acquired.

## EXPERIMENTAL

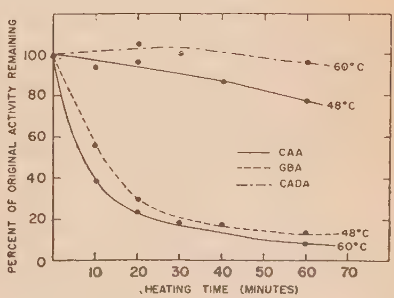
### *Rat liver*

Adult Sprague-Dawley rats of both sexes were sacrificed by decapitation. The livers were quickly removed, and homogenized with 2 volumes of ice water in the Waring blendor. After centrifuging for 90 minutes at 42,000g, the supernatant fluid was decanted and lyophilized. For the differential studies, the appropriate amount of lyophilized product was dissolved in water and adjusted to pH 6.2 with 0.1 M $KH_2PO_4$.

Dehydropeptidase activity was determined as described below except that here 0.2 M tris buffer at pH 8.0 was employed. Chloroacetyl-*L*-alanine hydrolysis was followed as usual by means of the Van Slyke manometric ninhydrin procedure; the digestion was conducted at pH 7.0 with phosphate buffer.

The results at 48° and 60° are shown in Figure 1, and clearly show that the

Figure 1
The differential heat denaturation of the activities in the soluble fraction of rat liver toward chloroacetyl-*L*-alanine (CAA), chloroacetyldehydroalanine (CADA), and glycyldehydroalanine (GDA).



activities toward chloroacetyl-*L*-alanine, chloroacetyldehydroalanine and glycyldehydroalanine are readily separable*.

### *Mouse liver and mouse hepatoma*

Female C3H mice about 6—7 months old were employed. The tumor was hepatoma 29 (originally induced by administration of carbon tetrachloride) at about the 29th subcutaneous passage in C3H mice, and at about the 20th day of growth. We are indebted to Dr. H. B. Andervont for this material. The animals were sacrificed by decapitation, and the tissues quickly removed and homogenized in the Potter-Elvehjem apparatus with four times the weight of cold 0.9% NaCl. Fractionation was accomplished by centrifugation at 105,000g for 75 minutes. Both supernatant and sediment were brought to the original volume of homogenate by addition of cold saline.

The digest consisted of 1 ml of enzyme preparation, 1 ml of 0.1 M borate at pH 8.2 and 1 ml of 0.025 M neutralized substrate. In control runs without substrate, the substrate was added at the end of the incubation period, the mixture treated with acetic acid, and dipped into a boiling water bath. After alkalinization, the ammonia was distilled into 2% boric acid and titrated with 0.05 N HCl. The data are given in Table II.

TABLE II
*Susceptibility of glycyldehydroamino acids to liver and hepatoma fractions*

| *Tissue* | *Fraction* | *Substrates* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Glycyldehydroalanine* | | *Glycyldehydronorvaline* | | *Glycyldehydrophenylalanine* | |
| | | *Hydrolytic rate*[1] | *% Total* | *Hydrolytic rate*[1] | *% Total* | *Hydrolytic rate*[1] | *% Total* |
| Mouse liver | Homogenate | 13.5 | — | 2.5 | — | 0.5 | — |
| | Supernatant | 35.4 | 90 | 3.8 | 68 | 0 | 0 |
| | Sediment | 2.5 | 10 | 1.0 | 32 | 0.5 | 100 |
| Mouse Hepatoma | Homogenate | 20.0 | — | 2.3 | — | 0 | — |
| | Supernatant | 56.0 | 96 | 6.2 | 95 | 0 | — |
| | Sediment | 1.5 | 4 | 0.2 | 5 | 0 | — |

1) Rate in terms of micromoles hydrolyzed per hour per mg N.

The increased activity toward glycyldehydroalanine in the hepatoma homogenate represents an actual increase in total enzyme activity of about 17% on a wet tissue weight basis. Still larger increases might be expected for the more malignant rat hepatomas. Although the changes noted in Table II are in the same direction as those previously observed[5], such data as are available for other types of hepatomas indicate a considerably larger increase in activity of the hepatomas toward glycyldehydroalanine than that revealed in the present instance. Mouse hepatomas are often benign in nature, and tend to maintain more of the normal levels of the tissue of origin than do other, more malignant, tumors.

* Attempts to separate the very labile soluble enzymes attacking glycyl *L*-alanine and glycyldehydroalanine by differences in their heat and pH stability have been dubious. Although definite differences in the relative rates of denaturation can be observed, so much cross specificity is evident that a clear-cut separation has been as yet impossible.

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# BIOREDUCTION OF TRIMETHYLAMINE OXIDE

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In 1851 large quantities of trimethylamine were first found in herring brine[1]. As source material such naturally occurring widely encountered substances as betaine, carnitine, choline, neurine, etc. have been considered. Its formation by putrefying bacteria was especially held probable. A microbial formation from tetramethylammonium hydroxide (tetramine), a closely related compound, was precluded[2] by the discoverers of this substance in nature[3]. Trimethylamine oxide represents a definite source for the amine. In 1909 this oxide was found as a component of the flesh of salt water fish, and was subsequently identified in the organs and secretions of many species of fish[4] and marine animals as well as in terrestrial animals[5]. An interaction of trimethylamine and its oxide was described in investigations[5] by Ackermann, Hoppe-Seyler, Lintzel, Polonovski and Degrez, Susuki, Suwa at al. Ackermann, Poller and Linneweh[6] recognized in 1926 that trimethylamine oxide may act as hydrogen acceptor and thus participate in oxido-reduction processes. The phenomena of phytochemical reduction in turn are linked to oxido-reduction processes. In spite of the variety of types of bioreductions, their action mechanism according to F. G. Fischer and Eysenbach[7] should be interpreted to the effect that the agents of the cozymase-flavin system assume the functions of the "Gaerungswasserstoff".

A comprehensive review[8] shows which nitrogen-oxygen compounds were found accessible to phytochemical reduction. They are essentially the nitro-, nitroso, hydroxylamino- and oxamino-derivatives, as established during the years 1914—1923. Bioreduction of the trialkylamine oxides of the type $(R_3)N{:}O$ had not been previously attempted. Suwa[4] reported that putrefaction results in a transformation. The microorganisms were not characterized, however, and the method of analysis was at that time inaccurate. The old familiar observation[9] that trimethylamine is formed in the ammonia fermentation of urine is adequately explained by the fact that 50 mg trimethylamine oxide are found in 1000 ml of normal human urine[5]. Tarr[10] found reduction effects in only 3 out of 30 different microorganisms (aerobic and anaerobic) isolated from fish. These conditions are of practical interest to the food industry with regard to the problem of spoilage of fresh fish. This is shown in a conclusive review by Reay[11]. The Canadian investigators Beatty, Collins, Tarr and Watson[12] assumed the concurrent effect of a triaminoxidase which activates the trimethylamine oxide rendering it reducible by dehydrogenase systems present. A variety of hydrogen donators might be involved, e.g. lactic acid, which is produced in all autolytic processes of tissues. The reaction is formulated as follows:

$$2(CH_3)_3NO + CH_3.CHOH.COOH = CO_2 + H_2O + CH_3.COOH + 2(CH_3)_3N.$$

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The formation of a volatile acid (acetic acid) is considered characteristic. However, conditions do not seem to be as simple as that. According to Shevan[13], a formation of dimethylamine definitely precedes production of trimethylamine. Its precursor and the reaction mechanism are unknown. If the transition of trimethylamine oxide into trimethylamine requires the presence of triaminoxidase, it might be considered whether the course of the reaction differs from that of the numerous well-known anaerobic phytochemical reductions[8]. Tomita and Tanaku[14] carried out in Japan an investigation on phytochemical production of trimethylamine from its oxide which had been started in Dahlem in 1930. They succeeded in isolating the tertiary amine; they used yeast, the yield obtained was fair.

In view of the previously mentioned role of saprophytes, the phytochemical reduction of trimethylamine oxide by means of a well characterized bacterium represents a certain interest. The reduction was carried out with *Pseudomonas Lindneri* (*Thermobacterium mobile*). A desmolytic system, identical to that of yeast, is available to this bacillus. It readily decomposes zymohexoses into $CO_2$ and ethanol, and its enzyme system includes carboxylase, phosphatases, ketonaldehydmutases, hydrolases, carboligase and others and is also endowed with phosphorylation capacity. All these data are described in the literature[15].

*Thermobacterium mobile* effects phytochemical reduction of trimethylamine oxide.

The experimental set-up was similar to that of Neuberg and Grauer[16]. It was necessary, however, to extend the duration of the experiments as well as to increase the quantities of bacteria and sugar, at the expense of which reduction of the aminoxide is effected. Determination of the resulting trimethylamine is facilitated by the exact method of Lintzel, Pfeiffer and Zippel[17]. According to these authors the quantity of amine oxide which escaped reduction may also be determined. It was shown that the quantity of amine oxide which disappeared coincided with that determined for trimethylamine and amounted to 67% of the theory.

The trimethylamine oxide dihydrate, $(CH_3)_3NO.2H_2O$, used in these experiments was prepared according to Meisenheimer.

To the sterile solution of 500 g glucose in 5000 ml $H_2O$, 500 ml bacterial suspension were added. The latter was obtained by a three day growth of the organism at 28°. After 2 hours of incubation at 28° strong $CO_2$ production was in progress. 2 g of magnesium fructose-6-phosphate were added since, according to Fischer and Eysenbach[7], this substance promotes the transfer of hydrogen. A solution of 11.1 g trimethylamine oxide dihydrate in 120 ml $H_2O$ was subsequently added from a separatory funnel over an 8 hour period. A noticeable interruption of the fermentation process did not occur. In a control test carried out with smaller quantities relatively high amounts of unchanged oxide were found after 3 days, while sugar had been transformed almost quantitatively. Therefore 250 g glucose in 10% solution as well as twice the quantity of the initially applied bacterial suspension as a fresh culture were added. After another three days the fermentation mixture was filtered. 3/4 thereof was acidified with 10% HCl and concentrated *in vacuo* at 30° to a volume of 200 ml in view of the high alcohol content. According to the above-mentioned method of determination[17], 3.95 g of trimethylamine and 3.57 g of unchanged oxide were found for the entire experimental quantity. The results as calculated on the basis of either value coincide satisfactorily and it was found that bioreduction had progressed to about 2/3 of the theoretical value. The resulting tri-

methylamine was identified as picrolonate, reineckate and by means of Mayer's reagent (i.e. Nessler's reagent free of alkali).

To the remaining quarter of the fermentation mixture NaOH was added to weakly alkaline reaction and it was concentrated to a volume of 250 ml. Upon acidification with diluted sulphuric acid, the volatile acid content was separated by steam distillation. Titration of the distillate showed insignificant amounts only which did not exceed the amounts obtained in control tests without trimethylamine oxide. There was no formation of volatile acid according to the previously cited oxido-reduction mechanism.

Under normal conditions of bioreduction, the reduction of the trimethylamine oxide does not proceed as smoothly as that of other nitrogen-oxygen compounds of different constitution. It should be mentioned, furthermore, that pyridine-*N*-oxide which is now readily accessible according to Ochiai[18] is reduced to pyridine to a moderate extent under identical conditions. Reduction in the pyridine ring proper has not been obtained.

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# NOTE ON THE AGEING OF BITUMEN

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When a specimen of bitumen is left to itself, it hardens in the course of time. This phenomenon is called *ageing*. While the term is sometimes used as synonymous with *weathering*, it should be distinguished from the latter, which is a much more severe and complex process, as reproduced in the "weatherometer" in which radiation, artificial rain, heat or cold are applied for any required period.**

Ageing proper occurs in the absence of light and severe atmospheric influences and at room temperature. It is of great practical importance in the performance of road asphalts.

A large literature exists on this subject. Part of it was surveyed by one of us (R. Sch.—R.[1]). Recently W. P. van Oort has added a systematic study in a published Ph. D. dissertation[2].

He distinguishes two kinds of hardening:

(i) *physical* hardening which is due to a change of structure in the bulk of bitumen;

(ii) *chemical* hardening which is due to oxidation. He describes the mechanism of oxidation as follows: oxygen is *dissolved* in the exposed bitumen surface. As a result of the difference in its concentration in the bitumen, the oxygen diffuses inwards. At the same time chemical reaction occurs at a velocity proportional to the local concentration of physically dissolved oxygen. As a result the bitumen hardens, which leads to a reduction of the diffusion coefficient. Gradually a film is formed of a thickness of the order of $10\,\mu$.

The purpose of the present note is to draw attention to a mechanism of chemical hardening which is of a very different character.

In the course of experiments by means of a co-axial rotating cylinder viscometer of the standard type[3] (undertaken for other purposes), we observed a gradual hardening of the bitumen from day to day. In order to examine the phenomenon more closely, we made systematic experiments with a heavily blown very elastic bitumen. Using a small load the internal cylinder was rotated, and the times noted until a constant very small rotation of 0.86° was reached, the observations being made by means of a mirror-telescope arrangement. This time-interval may be considered as a measure of the hardness or stiffness of the material (compare Figure 1). With a fresh sample that time was 1480 seconds. This gradually increased in the course of 22 days to 2955 seconds (Exp.

* May I be permitted to express my gratitude to Prof. Fodor, who in 1923 accepted the then young student from Berlin as his assistant at Halle. When Prof. Fodor moved in 1924 to Jerusalem, he took me with him, and I thus became one of the first scientific workers at the Hebrew University even before its formal opening. I had the honour to assist him until 1932 in his research work which resulted in ten scientific papers.

** Such a weathering machine is installed in the Standards Institution, where experiments are carried out by Dr. Yoffe.

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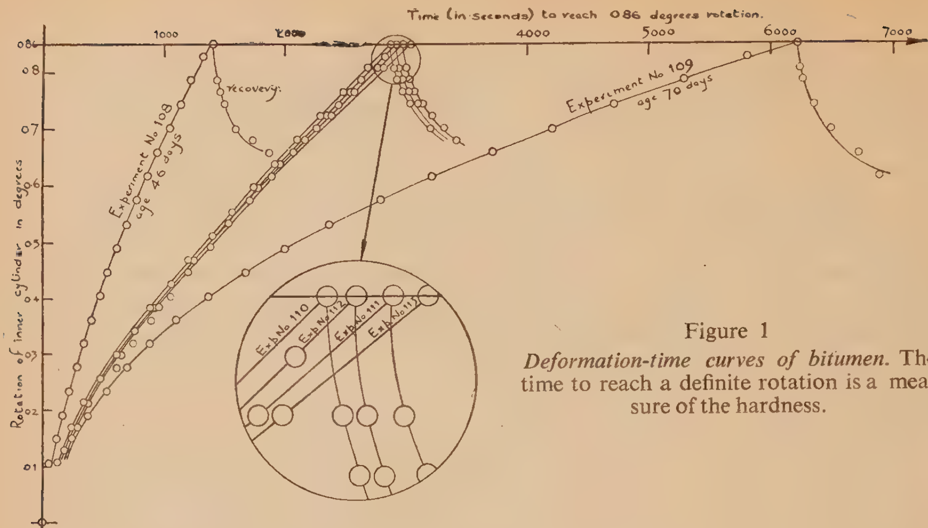


Figure 1
*Deformation-time curves of bitumen.* The time to reach a definite rotation is a measure of the hardness.

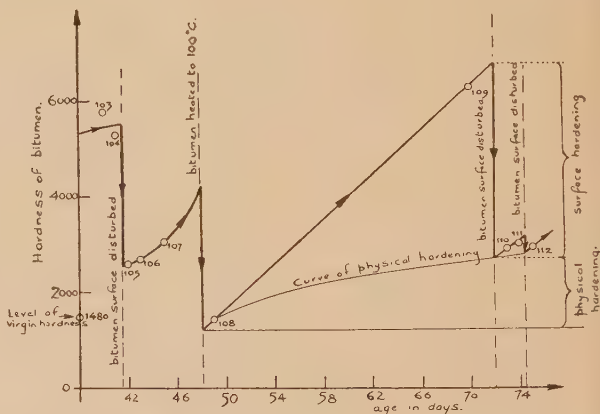


Figure 2
*Hardness—versus—age curves derived from the deformation curves shown in Figure* 1. The number written against each observation-point is the number of the experiment.

No. 93), equivalent to more than 100% age-hardening. The increase was not uniform: it took place first at a rate of about 20% per day, the rate gradually decreasing until an equilibrium was reached after 16 days. No appreciable age-hardening took place in the course of the next few days. The elastic recovery also increased during the same period, reaching nearly complete recovery after about ten days. On the 22nd day the top layer of bitumen, with which it covers the internal cylinder and which is about 1 mm thick, was removed. The hardness fell at once to about 2400 seconds (Exp. No. 94), which meant a loss of about half of its age-hardening, while the increase in elasticity through age was entirely lost. The experiment was then repeated. Now the top layer of the bitumen covered the annulus only, extending from the external to the internal cylinder. The hardness increased during a number of experiments reaching (Exp. No. 104) 5200 seconds after 17 days (compare Figure 2). Note that now the increase was more pronounced than in the first series. The bitumen surface was then disturbed by some slight manipulation which resulted in a drop of hardness to 2600 seconds. It eft a hardness of about 1100 seconds unaffected. After 7 days the hardness had

again increased. When the bitumen was now heated to 100°C, all hardness went entirely back to the virgin state (and even slightly below due to the removal of some of the bitumen, as mentioned above). For the rest the reader may compare the two figures.

It seems to us that these experiments show that in addition to the physical hardening taking place in the bulk of the bitumen, there is a pronounced *surface hardening*. This is not the type of weathering hardening described by van Oort, but is confined to an exceedingly thin skin which can be broken by the slightest disturbance. We suggest that this skin is a monomolecular surface film of oxidized material. While the type of weathering oxidation described by van Oort will slowly proceed in the course of time, it seems to us that on the road the surface film oxidation may be more important. What will happen in the life time of the asphalt road may be described as follows: a monomolecular surface film is formed on the bitumen when at rest between two contacts with the wheel. When a wheel breaks the film, the oxidized material making up the film is mixed into the body of the bitumen and another surface exposed. This process is continuously repeated until the whole of the bitumen consists of oxidized material with the accompanying ageing effects.

We are grateful to Prof. E. D. Bergmann with whom we had the privilege to discuss the problem.

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# NOTE ON THE ELASTICITY OF SOLID-SOLID DISPERSIONS

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The first and best known micro-rheological investigation, and one before the term rheology had been coined, leads to Einstein's equation

$$\eta = \eta_o(1 + 2.5\, c_v) \tag{1}$$ [1]

of the viscosity $\eta$ of a dilute dispersion of rigid spheres of volume-concentration $c_v$, in a liquid medium of viscosity $\eta_o$. An analogous problem is the one concerning the elasticity of a solid-solid dispersion, such as e.g. a cement mortar where the solid sand is dispersed in the solid cement or a filled rubber where the solid filler is dispersed in the rubber. Let $\mu$ denote the shear modulus, then, *if the cement were incompressible*, we would simply have

$$\mu = \mu_o(1 + 2.5\, c_v) \tag{2}$$

However, the cement has a finite modulus of compressibility $K_o$. The problem is therefore one degree more complicated than Einstein's. One of us (Z. Ch.) has solved the mathematics using spherical harmonics of negative integral degree[2]. Assuming that sand is incomparably more rigid than cement, the result is

$$\mu = \mu_o\left(1 + \frac{15}{2}\cdot\frac{1-\nu}{4-5\nu}c_v\right)$$

$$K = K_o\left(1 + 3\cdot\frac{1-\nu}{1+\nu}c_v\right) \tag{3}$$

where $\nu$ is Poisson's ratio of the dispersion medium. The dispersion medium is incompressible if $\nu = 1/2$. In this case Eq. (3.1) is reduced to (2). The details of mathematical development will be published elsewhere.

* May I be permitted to add a personal reminiscence concerning my scientific connection with Prof. Fodor. After he arrived in Jerusalem in 1924, we lived in the same Arab house in the so-called American Colony which is now in Jordan-Jerusalem. It was therefore natural that he, the chemist, came to discuss scientific questions with me, the civil engineer. Until then I had hardly heard of colloid chemistry, but then he drew my attention to a problem raised by Freundlich and Schalek in the *Zeitschrift für Physikalische Chemie*. This problem, which presented considerable difficulties to a chemist, was comparatively simple to a worker in the field of Mechanics of Materials. I wrote a paper which contained what is now generally known as the Buckingham-Reiner equation. Prof. Fodor was instrumental in having it published in the *Kolloid-Zeitschrift*. As a result of the recognition which the paper found in the United States, Prof. Bingham invited me to Lafayette College where we both, again he the chemist and I the "Mechanician", conceived the idea of a separate branch of physics extending over the border land between both of us and for which Prof. Bingham coined the name Rheology. Prof. Fodor thus had a hand in the formation of this flourishing branch of science.

M. R.

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# THE ACTION OF CHLOROMYCETIN, AUREOMYCIN AND TERRAMYCIN ON THE BIOSYNTHESIS OF INDOLE IN *ESCHERICHIA COLI*

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Though extensive work has been carried out on the mode of action of chloromycetin (chloramphenicol), aureomycin and terramycin, the point at which the compounds attack the normal metabolism of the bacterial cell is not yet clear.

Regarding the point of attack of chloromycetin, two hypotheses have been offered, both related to the biosynthesis of the aromatic amino acids. Woolley[1], using *Escherichia coli*, assumed that the antibiotic interferes with the utilisation of phenylalanine, due to the structural resemblance between the two compounds. Truhaut, Lambin and Boyer[2], on the other hand, suspected, in *Eberthella typhi*, blockage of the synthesis of tryptophan from anthranilic acid via indole[3,4,5,6].

In the case of aureomycin and terramycin, it has been stated only very recently[7] that no metabolites have been found which are capable of reversing the inhibition of these antibiotics.

The present investigation is devoted to a study of the effect of chloromycetin, aureomycin and terramycin on the biosynthesis of the aromatic amino acids in the wild strain of *E. coli* and its aromatic amino acids requiring mutants. In addition, the effect of 5-fluorotryptophan on these organisms has been compared with that of the antibiotics. A preliminary report of the results has been published elsewhere[8].

## EXPERIMENTAL

### *Cultures and media*

The cultures used were: *Escherichia coli*, wild type (ATCC 9637) and its mutants: anthranilic acid-indole-tryptophan mutant (121-35), designated as "anthranilic acid mutant", indoleless-tryptophanless mutant (19-2), phenylalanineless mutant (M83-5) and tyrosineless mutant (M83-9).

They were kept on agar mineral medium, as described by Davis and Mingioli[9], supplemented with 0.5% (v/v) of enzymatic casein hydrolysate (Nutritional Biochemical Corp.). The various bio-assays were carried out in the same mineral medium to which the required metabolites were added. The media were autoclaved at 15 pounds pressure for 15 minutes.

For inoculum, the surface growth from a 8 hr. agar slant was gently scraped off and suspended in sterile 0.9% (w/v) sodium chloride solution to a turbidity reading of

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56-60 on the Klett-Summerson photoelectric colorimeter, using a No. 64 red filter. From this heavy suspension, 0.3 ml was diluted into 10 ml sterile saline; each experimental culture was inoculated with a drop of this dilute suspension. The final volume of the media in all experiments was 2.5 ml. Cultures were incubated at 37° for 17 hrs., and growth turbidity was determined as above.

The growth of the wild type in the various cultures studied did not change at an incubation time of 24 hrs. In the case of the mutants, however, back-mutation occurred in some cultures after incubation for periods longer than 20 hrs.

Most of the compounds tested were added directly to the medium before its sterilization; the following ones were added aseptically as aqueous solutions which had been filtered through a glass filter: chloromycetin, aureomycin, terramycin, *p*-aminobenzoic acid and its *N*-formyl derivative, folic acid, leucovorin-calcium salt, authranilic acid and its derivatives, choline chloride, trimethyl phosphate and methyl alcohol.

### *Materials*

The amino acids, purines, pyrimidines and vitamins were products of the Nutritional Biochemical Corporation. Leucovorin-calcium (R 2154-173) was kindly supplied by Dr. Harry P. Broquist of the Lederle Laboratories, *o*-amino-acetophenone hydrochloride by Dr. C.E. Dalgleish and dimethyl-β-propiothetin hydrochloride by the California Foundation for Biochemical Research. Chloromycetin, aureomycin and terramycin were Parke Davis products; dihydro-streptomycin was a product of Pfizer Co. and bacitracin was obtained from Messrs. Commercial Solvents.

*N*-Formylanthranilic acid was prepared according to von Meyer and Bellman[10] (m.p. 165-168°), *N*-methylanthranilic acid, according to Houben and Brassert[11]. It crystallized from methyl-isobutylketone and glacial acetic acid (m.p. 178-180° (dec.)). Isatoic anhydride was prepared according to Ben-Ishai and Katchalski[12], and 5-fluorotryptophan following the directions of Rinderknecht and Niemann[13].

## RESULTS

### *Experiments with chloromycetin, aureomycin and terramycin*

*The point of attack.* The amounts of the antibiotics necessary to reduce the growth of *Esch. coli*, wild strain, to 50% of full growth, and to inhibit it completely, were determined from dose response curves. The following compounds tested on cultures in which growth was inhibited by 2 μg/ml of chloromycetin (yielding 45% of full growth), 2 μg/ml of aureomycin (65% of full growth) and 0.3 μg/ml of terramycin (40% of full growth) were found not to relieve the inhibition:

(a) *Amino acids* (amounts up to 2000 μg/ml): *DL*-alanine, anthranilic acid, *L*-arginine hydrochloride, *L*-aspartic acid, *L*-cysteine, *L*-glutamic acid, glutathione, glycine, *L*-histidine (which increased the growth by about 10%), *DL*-homocysteine, *L*-hydroxyproline, *DL*-isoleucine, *L*-leucine, *L*-lysine, *L*-proline, *DL*-serine, *DL*-threonine, *DL*-valine.

(b) *Vitamins*: *p*-aminobenzoic acid (up to 4 μg/ml), folic acid (up to 4 μg/ml), leucovorin-calcium salt (up to 100 μg/ml).

(c) *Purines and pyrimidines* (amounts up to 400 μg/ml): adenine sulphate, cytidine sulphate, uracil. (The effect on the aureomycin and terramycin inhibition was not tested).

In the case of chloromycetin, the inhibition determined at various concentrations of the inhibitor was partly reversed by indole and tryptophan, and only very slightly by phenylalanine and tyrosine, as shown in Table I ("A").

The inhibition by aureomycin can be reversed, within limits, by indole, tryptophan and, in a much lesser degree, by phenylalanine, and not at all by tyrosine (Table I, "B"); in the terramycin-inhibited organism the reversal is more effective, and the differences between the four metabolites are less pronounced (Table I, "C").

TABLE I

*Reversal by indole, tryptophan, phenylalanine and tyrosine of the inhibition of* E. coli, *wild type, by chloromycetin, aureomycin, terramycin and 5-fluorotryptophan.* (*Incubated for* 17 *hours at* 37°). (*Full growth* = *galvanometer reading G.R.* 100—110). (*Antibacterial index* = μg/ml *inhibitor:* μg/ml *metabolite, given at* 50 *and* 100% *growth*).

A. *Chloromycetin*

G.R. without metabolites
for: 1.6 μg/ml of chloromycetin = 60
for: 2.0 " " = 40
for: 2.4 " " = 25

| *Inhibitor* μg/ml. | *Metabolite* | μg/ml *required for* 50% *growth* (G.R. 55) | μg/ml *required for* 100% *growth* (G.R. 110) | *Antibacterial Index at* 50% *growth* |
|---|---|---|---|---|
| 1.6 | Indole | | 20—110 (85% growth) | |
| 2.0 | " | 12 | 20—110 (68% growth) | 0.16 |
| 2.4 | " | 20 | * | 0.14 |
| 1.6 | *DL*-tryptophan | | | |
| 2.0 | " | 200 | 400-2000 (68% growth) | 0.01 |
| 2.4 | " | 400-2000 (40% growth) | | |
| 1.6 | *DL*-phenylalanine | | | |
| 2.0 | " | 400 | * | 0.005 |
| 2.4 | " | * | | |
| 1.6 | *L*-tyrosine | | | |
| 2.0 | " | 400 | * | |
| 2.4 | " | * | | |

* No increase of growth above the indicated inhibition level could be obtained at concentrations up to 2000 μg/ml of the metabolite.

B. *Aureomycin*

G.R. without metabolites
for: 2.0 μg/ml of aureomycin = 65
for: 3.2 " " = 15

| *Inhibitor* μg/ml | *Metabolite* | μg/ml. *required for* 50% *growth* (G.R. 55) | μg/ml. *required for* 100% *growth* (G.R. 110) | *Antibacterial Index at* 50% *growth* | *Antibacterial Index at* 100% *growth* |
|---|---|---|---|---|---|
| 2.0 | Indole | | 40 | | 0.05 |
| 3.2 | " | 40-80 (max. 47% growth) | | 0.08 | |
| 2.0 | *DL*-tryptophan | | 400 (85% max. growth) | | |
| 3.2 | " | 400 | 2000 (65% max. growth) | 0.008 | |
| 2.0 | *DL*-phenylalanine | | 50-400 (70% max. growth) | | |
| 3.2 | " | 50-400 | * | 0.06 | |
| 2.0 | *L*-tyrosine | | * | | |
| 3.2 | " | 400 (max. 20% growth) | * | | |

* No increase of growth above the indicated inhibition level could be obtained at concentrations up to 2000 μg/ml of the metabolite.

C. *Terramycin*

G.R. without metabolites
for: 0.2 μg/ml of terramycin = 70
for: 0.3 " " = 40

| *Inhibitor μg/ml* | *Metabolite* | *μg/ml required for* 50% growth (G.R. 55) | 100% growth (G.R. 110) | *Antibacterial Index at* 50% growth | 100% growth |
|---|---|---|---|---|---|
| 0.2 | Indole | | 12 | | 0.15 |
| 0.3 | " | 20 | 40 (max. growth 75%) | 0.15 | |
| 0.2 | *DL*-tryptophan | | 40 | | 0.05 |
| 0.3 | " | 60 | 400 | 0.05 | |
| 0.2 | *DL*-phenylalanine | | 40 | | 0.05 |
| 0.3 | " | 20 | 200 | 0.15 | |
| 0.2 | *L*-tyrosine | | 40 | | 0.05 |
| 0.3 | " | 40 | 400 (max. growth 75%) | 0.07 | |

D. *5-Fluorotryptophan*

G.R. without metabolites
for: 0.1 μg/ml of 5-fluorotryptophan = 0
for: 10.0 " " = 0

| *Inhibitor μg/ml* | *Metabolite* | *μg/ml required for* 50% growth (G.R. 55) | 100% growth (G.R. 110) | *Antibacterial Index at* 50% growth | 100% growth |
|---|---|---|---|---|---|
| 0.1 | Indole | 0.01 | 0.04 | 10 | 2.5 |
| 10.0 | " | 0.2 | 0.4-100 (max. growth 75%) | 50 | |
| 0.1 | *DL*-tryptophan | * | 0.04 | | 2.5 |
| 10.0 | " | ** | 0.4 | | 25 |
| 0.1 | *DL*-phenylalanine | 0.5 | 0.8 | 0.2 | 0.12 |
| 10.0 | " | 2-2000 (max. growth 40%) | | | |
| 0.1 | *L*-tyrosine | 0.02 | 0.2 | 5 | 0.5 |
| 10.0 | " | 0.4-400 (max. growth 25%) | | | |

* 0.01-0.03 μg/ml of *DL*-tryptophan did not reverse the inhibition.
** 0.1-0.3 μg/ml of *DL*-tryptophan did not reverse the inhibition.

As anthranilic acid does not reverse the inhibition caused by any of the three antibiotics (up to 200 μg/ml), it appears that the compounds block the metabolism between anthranilic acid and indole.

The interference of the antibiotics with the utilization of the aromatic amino acids was tested in the inhibited mutants. Figures 1—4 show that the inhibitions in the indoleless, tryptophanless, phenylalanineless and tyrosineless mutants are reversed, to various degrees, by the corresponding amino acids. The antibacterial indices calculated from the dose response curves for various quantities of the inhibitor at 50% of full growth, are given in Table II.

TABLE II

*Antibacterial indices for the indoleless, tryptophanless, phenylalanineless and tyrosineless mutants of* E. coli, *inhibited by: chloromycetin, aureomycin, terramycin and 5-fluorotryptophan* (*incubated* 17 *hrs at* 37°)

| *Mutants* | *Chloromycetin* | | | *Aureomycin* | | | *Terramycin* | | | *5-Fluorotryptophan* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Inhibitor μg/ml* | *Metabolite μg/ml* | *Antibacterial Index* | *Inhibitor μg/ml* | *Metabolite μg/ml* | *Antibacterial Index* | *Inhibitor μg/ml* | *Metabolite μg/ml* | *Antibacterial Index* | *Inhibitor μg/ml* | *Metabolite μg/ml* | *Antibacterial Index* |
| Indoleless | 1.2 | 0.8 | 1.5 | 0.4 | 0.7 | 0.57 | 0.2 | 1 | 0.2 | 0.3 | 0.6 | 0.5 |
| | 2.0 | 2 | 1 | 1.2 | 5-40 (15% growth) | | 0.14 | 20-40 (15% growth) | | 0.4 | 0.7 | 0.57 |
| | 2.4 | 8 | 0.3 | | | | | | | | | |
| Tryptophanless | 1.2 | 20 | 0.6 | 0.4 | 0.4 | 1 | 0.2 | 1.5 | 0.13 | 0.6 | 0.6 | 1 |
| | 2 | 50-2000 (30% growth) | | 1.2 | 400 | 0.03 | 0.4 | 2000 (46% growth) | | 0.8 | 0.8 | 1 |
| Phenylalanineless | 1.2 | 9 | 0.13 | 0.4 | 6 | 0.06 | 0.2 | 5 | 0.04 | 0.2 | 4 | 0.05 |
| | 2 | 8-2000 (30% growth) | | 1.2 | 20 | 0.06 | 0.3 | 8 | 0.037 | 0.4 | 6 | 0.06 |
| Tyrosineless | 1.2 | 20 | 0.6 | 0.4 | 4 | 0.10 | 0.2 | 30 | 0.006 | 0.2 | 4 | 0.05 |
| | 2 | 40-400 (40% growth) | | 1.2 | 20 | 0.06 | 0.3 | 50-400 (15% growth) | | 0.4 | 6 | 0.06 |

* (Antibacterial index = μg/ml inhibitor: μg/ml metabolite; data calculated at 50% growth).

In the case of the anthranilic acid mutant, the inhibition by chloromycetin is only partially reversed by anthranilic acid at the lowest levels of the antibiotic. Increasing concentrations of anthranilic acid alone have an inhibiting effect (Figure 5). The inhibition of this mutant by aureomycin and terramycin is practically not reversed by anthranilic acid (Table IV, "B").

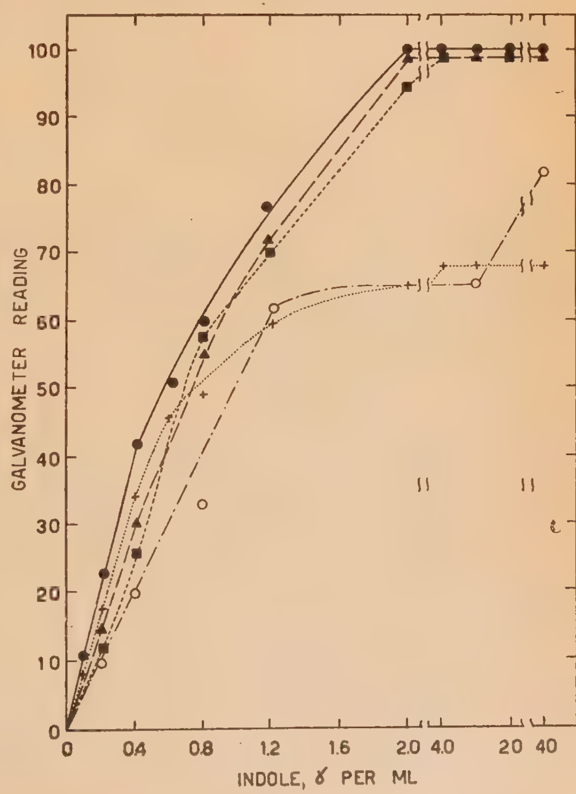


Figure 1

*Effect of chloromycetin, aureomycin, terramycin and 5-fluorotryptophan on growth of* Escherichia coli, *indoleless mutant.*

(●—●) indole alone. (+....+) addition of 1.2 μg/ml of chloromycetin. (▲—▲) addition of 0.4 μg/ml of aureomycin. (O—·—O) addition of 0.2 μg/ml of terramycin. (■—■) addition of 0.4 μg/ml of 5-fluorotryptophan (Incubated for 17 hrs at 37°).

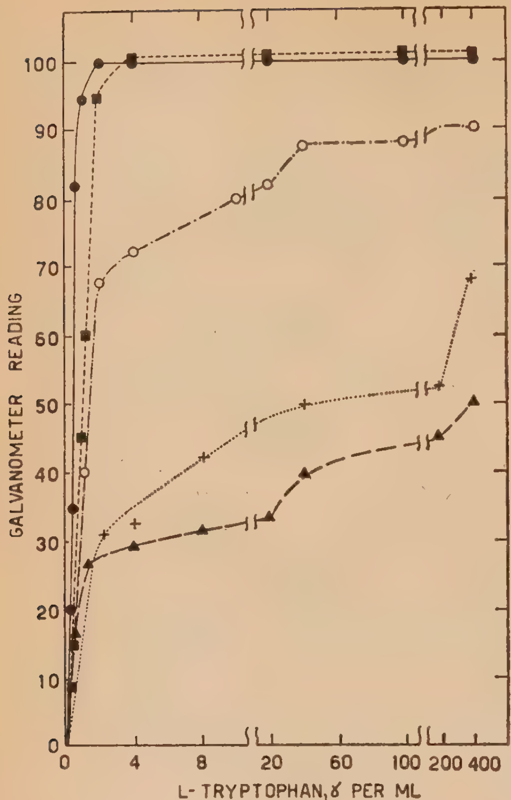


Figure 2

*Effect of chloromycetin, aureomycin, terramycin and 5-fluorotryptophan on growth of* Escherichia coli, *tryptophanless mutant.* (●—●) *L*-tryptophan alone. (+...+) addition of 1.2 μg/ml of chloromycetin. (▲—▲) addition of 1.2 μg/ml of aureomycin. (O---O) addition of 0.2 μg/ml of terramycin. (■--■) addition of 0.8 μg/ml of 5-fluorotryptophan (incubated for 17 hrs at 37°).

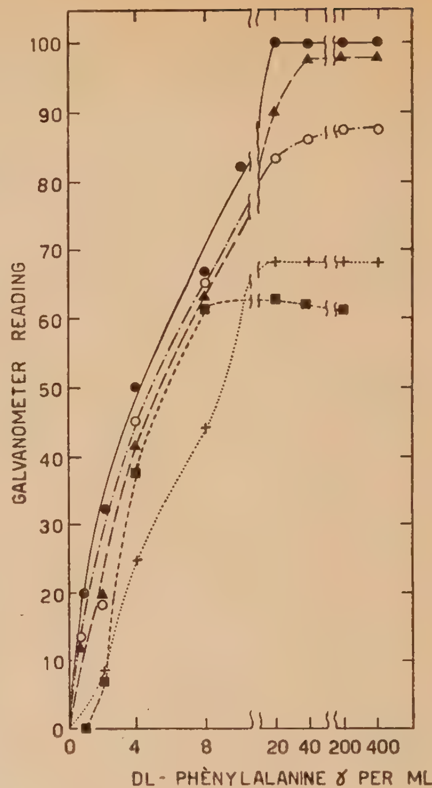


Figure 3

*Effect of chloromycetin, aureomycin and 5-fluorotryptophan on growth of* Escherichia coli, *phenylalanineless mutant.* (●—●) *DL*-phenylalanine alone. (+...+) addition of 1.2 μg/ml of chloromycetin. (▲—▲) addition of 0.4 μg/ml of aureomycin. (O---O) addition of 0.2 μg/ml of terramycin. (■--■) addition of 0.4 μg/ml of 5-fluorotryptophan (incubated for 17 hrs at 37°).

*Mechanism of indole formation.* The conversion of anthranilic acid into indole requires — at least formally — the participation of a $C_1$ compound. In order to determine its nature and specificity, a number of experiments were carried out with the chloromycetin inhibited wild strain of *Esch. coli.* The inhibited organism did not respond significantly to *N*-formylanthranilic acid (up to 40 μg/ml) (in a concentration of 0.4 μg/ml, a 15% increase in growth was observed), and not at all to *N*-methylanthranilic acid (up to 80 μg/ml), isatoic anhydride (up to 40 μg/ml) and *o*-amino-acetophenone hydrochloride (up to 400 μg/ml). A number of the usual methyl donors also proved ineffective alone or when applied in conjunction with anthranilic acid: sodium formate (up to 4 μg/ml), dimethyl-β-propiothetin hydrochloride (up to 40 μg/ml), choline chloride (40 μg/ml), betaine (40 μg/ml), methyl alcohol (2 μg/ml) and trimethyl phosphate (2 μg/ml) were inactive.

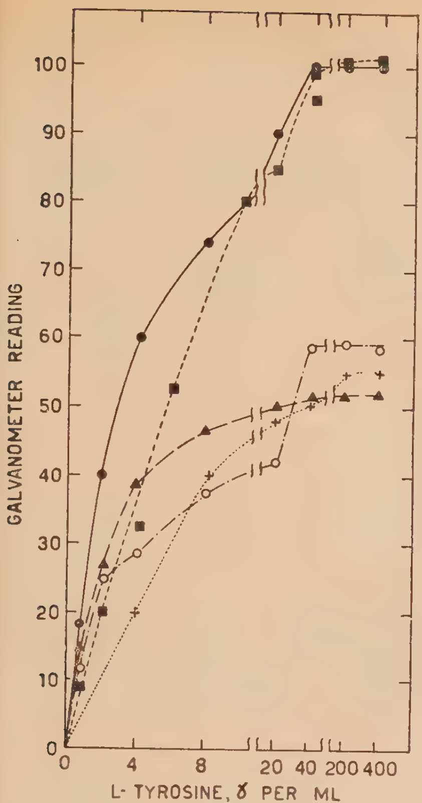


Figure 4

*Effect of chloromycetin, aureomycin, terramycin and 5-fluorotryptophan on growth of* Escherichia coli, *tyrosineless mutant.* (●—●) *L*-tyrosine alone. (+...+) addition of 1.2 μg/ml of chloromycetin. (▲—▲) addition of 1.2 μg/ml of aureomycin. (○——○) addition of 0.2 μg/ml of terramycin. (■—■) addition of 0.4 μg/ml of 5-fluorotryptophan (incubated for 17 hrs at 37°).

The inactivity of these substances was not affected by vitamin $B_{12}$ (0.0004 μg/ml). Methionine was the only substance which showed a positive effect at an optimal quantity of 12 μg/ml; this relief of the inhibition by chloromycetin increased when the amino-acid was given in conjunction with anthranilic acid and vitamin $B_{12}$ (Table III). $B_{12}$, which, alone, also showed a small reversing effect on the inhibition, could not be replaced by either *p*-aminobenzoic or folic acid.

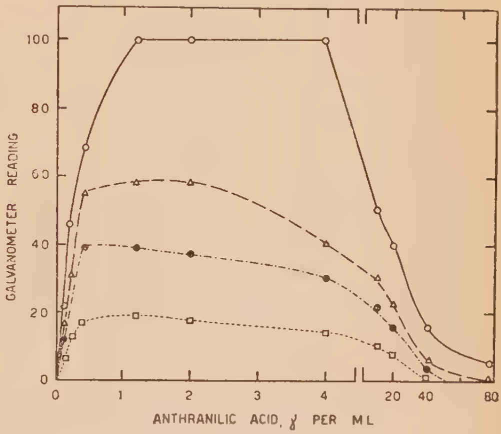


Figure 5

*Reversal of chloromycetin inhibition by anthranilic acid in* Escherichia coli *mutant requiring anthranilic acid.* (○—○) anthranilic acid alone. (▲---▲) addition of 1.2 μg/ml of chloromycetin. (●---●) addition of 1.6 μg/ml of chloromycetin. (■...■) addition of 2.0 μg/ml of chloromycetin (incubated for 17 hrs at 37°).

Identical observations were made in the *anthranilic acid mutant*. The inhibition by chloromycetin could be overcome, within limits of the inhibiting concentrations, by vitamin $B_{12}$ or methionine. The combination of anthranilic acid, methionine and vitamin $B_{12}$ was most effective. In the case of aureomycin and terramycin inhibition, too, methionine counteracted the effect within limits of the inhibitor concentration. Vitamin $B_{12}$ showed a very slight effect only in the case of terramycin (Table III).

It is interesting that the combination of homocysteine (20 μg/ml) and vitamin $B_{12}$ (0.0004 μg/ml) was also active (increase of growth from galvanometer reading 50 to 70), when given to the culture treated with chloromycetin (1.6 μg/ml) and anthranilic acid (0.4 μg/ml), whilst homocysteine alone had no effect. Nor was any effect obtained in the inhibited anthranilic acid mutant by adding any of the various amino acids, vi-

TABLE III

*Inhibition of* E. coli, *wild type, by chloromycetin, aureomycin, terramycin and 5-fluorotryptophan, and reversal by anthranilic acid,* DL-*methionine and Vitamin* $B_{12}$. (*Incubated* 17 *hrs. at* 37 °) (*G.R.* = *galvanometer reading*)

| | Inhibitor alone | | (1) Addition of anthranilic acid (4μg/ml) | (2) Addition of $B_{12}$ (0.0004 μg/ml) | (3) Addition of DL-methionine (12μg/ml) | (4) Addition of (1+2) | (5) Addition of (1+3) | (6) Addition of (2+3) | (7) Addition of (1+2+3) |
|---|---|---|---|---|---|---|---|---|---|
| | μg/ml | G.R. | G.R. | G.R. | G.R. | G.R. | G.R. | G.R. | G.R. |
| Chloromycetin | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | 1.6 | 50 | 51 | 55 | 63 | 54 | 65 | 68 | 74 |
| | 2.0 | 45 | 45 | 52 | 65 | 50 | 73 | 70 | 75 |
| | 2.4 | 33 | 34 | 42 | 47 | 41 | 52 | 56 | 60 |
| | 2.8 | 15 | 17 | 18 | 21 | 17 | 20 | 22 | 27 |
| Aureomycin | 2.0 | 65 | 72 | 70 | 91 | — | — | 91 | 110 |
| | 3.2 | 12 | 14 | 19 | 34 | — | — | 39 | 26 |
| Terramycin | 0.2 | 70 | 72 | 79 | 84 | — | — | 88 | 108 |
| | 0.3 | 49 | 43 | 48 | 62 | — | — | 70 | 78 |
| 5-Fluorotryptophan | 0.1 | 0 | 2 | 11 | 99 | 10 | 20 | 105 | 27 |

TABLE IV

*Inhibition of* E. coli, *anthranilic acid mutant, by chloromycetin, aureomycin, terramycin and 5-fluorotryptophan, and reversal by anthranilic acid,* DL-*methionine and vitamin* $B_{12}$. (*Incubated* 17 *hrs at* 37 °) (*G.R.* = *galvanometer reading*).

| | (1) Anthranilic acid alone | | (2) (1)+Inhibitor | | (3) Inhibitor* + μg/ml anthranilic acid elevating growth to that of Col. (1) | (4) (1)+Inhibitor* +12 μg/ml +DL-methionine | (5) (1)+Inhibitor* +0.0004 μg/ml $B_{12}$ | (6) (1)+Inhibitor*+ 0.0004 μg/ml $B_{12}$+12 μg/ml DL-methionine |
|---|---|---|---|---|---|---|---|---|
| | μg/ml | G.R. | μg/ml | G.R. | | | | |
| Chloromycetin | 0.2 | 48 | 1.2 | 35 | 0.3 | 42 | 42 | 45 |
| | 0.4 | 65 | 1.2 | 52 | no reversion** | 60 | 64 | 68 |
| | 1.2 | 95 | 1.2 | 58 | " " | 69 | 65 | 78 |
| | 4.0 | 105 | 1.2 | 55 | " " | 71 | 68 | 79 |
| Aureomycin | 1.2 | 82 | 2.0 | 30 | no reversion** | 58 | 58 | 51 |
| | 2.0 | 105 | 2.0 | 44 | " " | 68 | 76 | 75 |
| | 4.0 | 105 | 1.2 | 72 | " " | 78 | 92 | 88 |
| Terramycin | 0.4 | 56 | 0.2 | 42 | 1.2 | 54 | 49 | 57 |
| | 1.2 | 85 | 0.2 | 56 | no reversion** | 82 | 68 | 78 |
| | 2.0 | 100 | 0.2 | 58 | " " | 81 | 69 | 79 |
| 5-Fluoro-tryptophan | 0.4 | 56 | 0.04 | 30 | 1.2 | 58 | 45 | 59 |
| | 1.0 | 73 | 0.04 | 35 | no reversion** | 70 | 35 | 72 |
| | 2.0 | 95 | 0.04 | 45 | " " | 102 | 65 | 103 |

* The various quantities of the inhibitor used were those indicated in column (2). In the absence of the inhibitor, neither *DL*-methionine nor $B_{12}$ added, alone or combined, showed any effect on growth in the presence or the absence of the metabolite.

** Up to 40 μg/ml of anthranilic acid; larger quantities of the latter alone cause inhibition.

tamins and methyl donors tested in the wild strain. *N,N*-Dimethyl-*p*-aminobenzoic acid, also, was found to be inactive. *N*-Formyl- and *N*-methyl-anthranilic acids, which were utilized by the mutant in a manner similar to that of anthranilic acid, behaved like the latter in reversing the chloromycetin inhibition of the anthranilic acid mutant.

Even the inhibition of the mutant that was caused by an excess of anthranilic acid in the presence of chloromycetin (Figure 5) could be reversed only within certain limits, by means of methionine alone, and somewhat more effectively in combination with $B_{12}$: growth inhibition of 80 and 95%, caused by 20 and 40 μg/ml of anthranilic acid with 1.2 μg/ml of chloromycetin, respectively, was reduced to 50 and 84% by 12 μg/ml of methionine; addition of 0.0004 μg/ml of the vitamin caused an additional 10% of increase in growth.

### *Experiments with other antibiotics*

The observations on the action of chloromycetin on the wild strain and the mutants of *Esch. coli* encouraged the carrying out of comparative experiments with other antibiotics. Bacitracin had no effect on *Esch. coli*, wild type, even in quantities of 4000 μg/ml, and only at 6000 μg/ml did a slight reduction of growth appear (see Johnson, Anker, Scudi and Goldberg)[13a]. Dihydrostreptomycin, on the other hand, inhibited the growth of the organism completely in quantities of 0.4 μg/ml; smaller doses failed to show any inhibition (compare, for streptomycin, Murray, Paine and Finland[30]). Anthranilic acid (up to 40 μg/ml), indole (up to 40 μg/ml) and up to 2000 μg/ml of *L*-tryptophan, *DL*-phenylalanine, *L*-tyrosine or *DL*-methionine did not reverse this effect.

### *Experiments with 5-fluorotryptophan*

In view of the close connection between the antibiotic action and the inhibition in the anthranilic acid — indole — tryptophan system which the above results have established, similar experiments have been carried out with 5-fluorotryptophan. The analogy of the response of *Esch. coli* (wild type and mutants) to this substance and to chloromycetin, aureomycin or terramycin is practically complete. Unlike, however, in the case of the other inhibitors, anthranilic acid added together with methionine or with methionine plus vitamin $B_{12}$ to the 5-fluorotryptophan-inhibited *Esch. coli*, wild type, suppressed the reversing effect of methionine or its combination with $B_{12}$ (Table III). In this case, too, the point of attack lies in the conversion of anthranilic acid into indole. Quantitatively, the effectiveness of the fluoro-compound is much greater than that of the antibiotics. In the wild strain as little as 0.1 μg/ml suffices to inhibit growth completely, whilst 0.3 μg/ml of terramycin give a 60% reduction in growth, 2.0 μg/ml of chloromycetin a 55% and 2.0 μg/ml of aureomycin a 40% reduction. Quantitative data on the experiments are given in Tables I ("D"), II, III and IV and in Figures 1—4.

## DISCUSSION

The present study has shown that the inhibition of *Esch. coli*, wild type, by chloromycetin, aureomycin and terramycin can be reversed within limits by indole and tryptophan, and less by phenylalanine and tyrosine (the action of aureomycin is not reversed by tyrosine), but not by anthranilic acid. Similarly, the inhibition of the utilization o the metabolites caused in the indoleless, tryptophanless, phenylalanineless, tyrosineles s

and anthranilic acid-less mutants of *Esch. coli*, is reversed in various degrees by the respective metabolites.

These results tend to show that the antibiotics interfere with the conversion of anthranilic acid to indole. Similar effects of chloromycetin on *Eberthella typhi* and an analogous explanation have previously been reported by Truhaut, Lambin and Boyer[2]; this conclusion differs from the theory offered by Woolley[1] in which chloromycetin, in *Esch. coli*, interferes with the utilization of phenylalanine. The statements made by Molho and Molho-Lacroix[14] that chloromycetin inhibition in *Esch. coli* was not relieved by tryptophan, phenylalanine or methionine and that cysteine showed a synergistic effect, and by Mentzer, Meunier, Molho-Lacroix and Billet[15] that the inhibition was relieved by glycine, could not be confirmed for the strains of *Esch. coli* studied.

In the study of the reversal, by anthranilic acid, of the inhibition caused by chloromycetin, aureomycin and terramycin in the anthranilic acid mutant, it was found that this reversal is only partial and occurs only for a limited range of concentrations of the amino acid; larger concentrations of anthranilic acid inhibit the growth of the mutant. The interpretation of this phenomenon is a matter of speculation.

The skeleton of indole and that of anthranilic acid differ by one carbon atom; *the origin of this carbon atom can be traced to methionine* which reverses, within limits, the inhibition caused by the antibiotics both in the wild type and in the anthranilic acid mutant, and relieves partially the inhibition caused in the latter by large quantities of anthranilic acid in the presence of chloromycetin. None of the other well-known mehtyl-donors have been found active; *N*-formyl- and *N*-methyl-anthranilic acid were also inactive in this respect. The efficiency of methionine is enhanced by catalytic quantities of vitamin $B_{12}$, which itself has some reversing effect on the inhibited wild strain and on the anthranilic acid mutant of *Esch. coli*. These results closely parallel the observations made in the study of the biosynthesis of the purine skeleton in the same microorganism[16,17]; there, the step from 4-aminoimidazole-5-carboxamide to the complete skeleton of the purine is made possible by methionine, aided by catalytic quantities of *p*-aminobenzoic acid (in the present case, *p*-aminobenzoic as well as folic acid are inactive), and vitamin $B_{12}$ enhances the utilization of the imidazole-carboxamide by the purineless mutants of *Esch. coli*. In general, it is becoming increasingly clear that methionine plays an important role as a source of $C_1$ units. Thus, the *N*-methyl of nicotine[18,18a] and of hordenine[19,20], the *N*- and *O*-methyl of ricinine and the methylenedioxy-group of protopine[21] are derived from that amino acid.

The function of vitamin $B_{12}$ as a possible mobilizer of methyl groups is also demonstrated by the observation that the inhibition of the anthranilic acid mutant of *Esch. coli* by chloromycetin can be relieved by the combination of *homocysteine* with the vitamin.

It should be mentioned that, according to Nyc, Mitchell, Leifer and Langham[22], the carboxyl group of anthranilic acid does not appear in the indole nucleus in *Neurospora crassa*. As, however, there are undoubtedly differences in the metabolic processes in *Neurospora* and *Esch. coli*, for which no analogous experiments have yet been made, this point has not been taken into consideration in the above discussion.

The comparative study of the action of other antibiotics on *Esch. coli* has shown that bacitracin is inactive, and that dihydrostreptomycin is a very powerful inhibitor, for which no antidote has yet been found. The close similarity in the mode of action of chloromycetin, aureomycin and terramycin may be biochemically related to the pheno-

menon of "cross-resistance" existing for these compounds: a microorganism which has acquired resistance to chloromycetin has also become resistant to aureomycin and terramycin, and *vice versa*[23–28a]. On the other hand, there is no cross-resistance between these antibiotics and streptomycin[24,29,30]. Also the morphological changes brought about in *Esch. coli* by aureomycin, terramycin and chloromycetin are similar, and are different from those produced by streptomycin[31].

The conclusion drawn from the experiments reported here that the blockage caused by the three antibiotics occurs in the neighbourhood of tryptophan, is paralleled by the observations made with its 5-fluoro-derivative; it is remarkable that the antibacterial potency of the latter, *in vitro*, far exceeds that of aureomycin, terramycin and chloromycetin.

A variety of effects on enzymatic systems has been described for chloromycetin (for a review, see Smith[32]), and it is difficult to say whether they are all of primary nature or not. Wyss[33] has emphasized that quite possibly "more than one enzyme can be interfered with by a single metabolite analogue", such as the three antibiotics studied here. In any event, the attractive theory that chloromycetin owes its activity to the amide linkage in its molecule, cannot explain the closely analogous activity of aureomycin and terramycin which are naphthacene derivatives[34,35,36].

When this investigation had been completed, a paper by Foster and Pittillo[37] appeared, in which it is reported that the inhibition of *E. coli*, caused by aureomycin, chloromycetin, dihydrostreptomycin, terramycin and penicillin G, can be reversed by a number of amino acids, vitamins and purines, the most active compounds being riboflavin, lumichrome, inosine and glycine in the case of chloromycetin. The authors conclude that aureomycin and terramycin inhibit the synthesis of riboflavin. The present investigation gives a broader picture of the situation; in addition, it should be pointed out that the experimental procedures employed by Foster and Pittillo differ significantly from those used in the present study.

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# ON SPONTANEOUS MALIGNANT GROWTHS IN THE LEVANT VOLE (*MICROTUS GUENTHERI* D.A.)

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During a period of more than twenty years breeding the Levant vole, *Microtus guentheri*, in the laboratory, a number of spontaneous malignant tumours came to our notice. It has been only during the last eight years, however, that we have sent all cancer suspects to the Cancer Department of the Hebrew University for the autopsy and histological analysis of the tumours. We are grateful to Dr. A. Back and especially to Dr. A. Rosin for carrying out this work.

The overwhelming majority of our voles were sacrificed at a very early age for experimental purposes. None of the voles which died a natural or accidental death or were sacrificed for various reasons were autopsied. Thus, it is highly probable that a number of neoplasms were overlooked. After 1945, however, conspicuous mammary tumours were not overlooked. In some cases experimental transmission of the cancer to other individuals was attempted, but did not succeed.

Since the notes and slides concerning the three cases observed by Dr. Back between 1945 and 1947 have been left on Mt. Scopus and are not yet available, we can merely state that three cases of malignant tumours were confirmed. In 1949 we had to build up our stock from freshly caught voles, which are thus field stock or first generation in our breedings and malignant tumours in this material are certainly not incidental to domestication. Of the following six cases, five were studied by Dr. Rosin and full histological analysis is available. With one exception (Female 3378) they were mammary carcinomas. All individuals were females, all were over 600 days old, except Female 3378, the daughter of Female 2507, the only case of a non-mammary carcinoma. Of the five other tumours, one was in a virgin, a second one in a paired sterile female, the three others in multiparae.

Against a physiological life-expectancy of about 718 days, the ecological life expectancy of the Levant vole in nature is only 62 days and less than five percent of individuals survive 150 days. Considering the high age at which the tumours appear in our breeding, it is obvious that there will be almost no chance of finding a spontaneous cancer in wild field populations. These data give a picture of the potential role of cancer in wild rodent populations provided individuals would reach an old age. The six cases after 1949 were from laboratory bred animals where less than 40 females reached an age of about 500 days, i.e. an incidence of 15% mammary cancers and other malignant tumours recognisable by inspection. We have no reason to doubt that internal tumours also occur, or to assume that the cancer incidence in voles is higher than in other wild rodents, and we may infer that in a population in which life

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expectancy were prolonged by the elimination of other causes of death the cancer incidence would rapidly increase. Additional information on vole populations in Israel and their biology has been published[1,2].

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CASE LIST (SINCE 1950)

| | |
|---|---|
| Female 2088 | 639 days old. Has had 11 litters with a total of 38 young. On 23.6.50 with small nut-sized tumour on breast, grown on 2.7.50, the day of the dissection, to the size of a large nut. Dr. A. Back: malignant mammary tumour. |
| Female 2465 | 645 days old. Has had one litter of 4 young. Of two tumours the bigger one was in the left armpit, the smaller on the right breast at the height of the last rib. Both tumours were tubulopapillary adenocarcinoma of the mammae (11.11.51, Dr. Rosin). |
| Female 2507 | 745 days old. Has had 7 litters with a total of 18 young. Many ventral tumours, four of them between mandibula and armpit, four in the inguineal region; dorsad a small tumour beneath the left scapula. Microscopically they showed the structure of an alveolary adenocarcinoma of the mammae, and a lymphatic metastase (11.3.52, Dr. Rosin). |
| Female 3378 | 330 days old (daughter of Female 2507), unpaired virgin. A big tumour ($4 \times 3 \times 2$ cm) in the left inguineal region. The left femur is entirely enclosed into the tissue of the tumour. Microscopically: a mixed cell sarcoma with penetration into the bone and destroying it. No metastases in other organs (22.1.52, Dr. Rosin). |
| Female 3421 | 842 days old. Has had 5 litters with a total of 8 young (four times one young each only). A cherry sized tumour above the sternum and two small ones adhering to the breast above the tip of the right lung. The big tumour above the sternum is microscopically an alveolar adenocarcinoma of the mammae. Metastases of the same histological structure are found in the right lung (16.2.53, Dr. Rosin). |
| Female 4312 | 731 days old. Paired for over 600 days and having given no birth. A bean-sized tumour in the left armpit. No metastes, Adeno-carcinoma starts from mamma-tissue which is entirely destroyed (2.2.54, Dr. Rosin). |

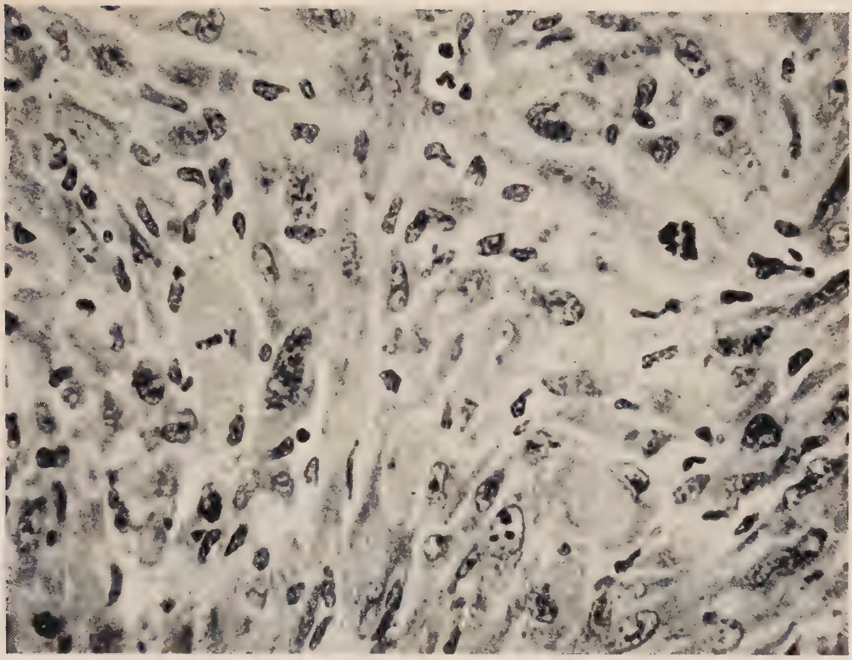

Figure 1

Microtus 3378. Mixed cell sarcoma × 560

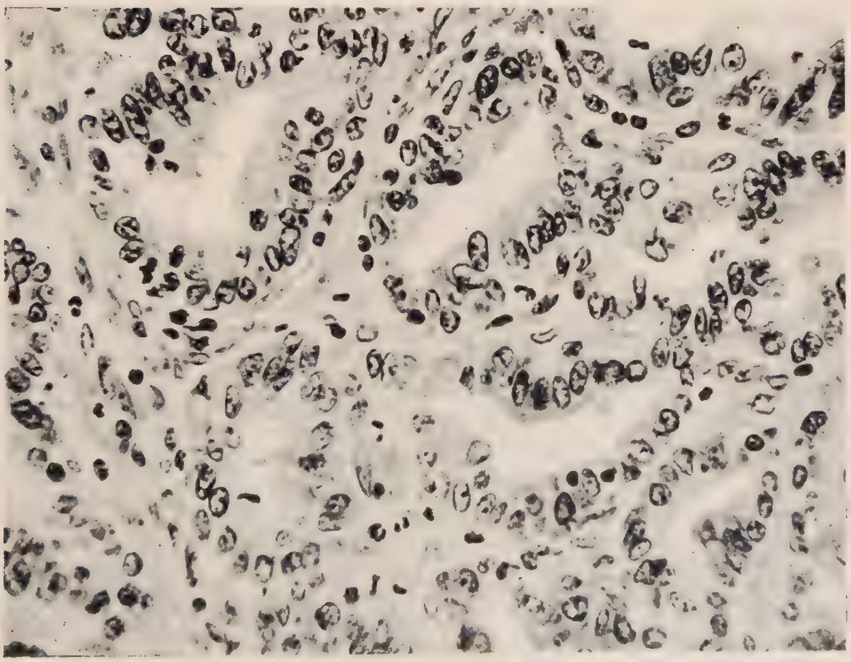

Figure 2

Microtus 2507. Adeno-carcinoma of mammary gland × 560

The photomicrographs are made from sections stained with Haematoxylin-Eosin.

# CATALYTIC EFFECT OF INORGANIC SALTS IN THE HETEROGENEOUS OXIDATION OF ALICYCLIC COMPOUNDS BY POTASSIUM PERCHLORATE

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In a previous communication[1] we described the aromatization of carbocyclic compounds by solid potassium perchlorate, carried out in sealed tubes at temperatures of 370 — 380°. The most attractive feature of this new aromatization method was that methyl and hydroxy groups were retained unaffected in the aromatization product. This fact gave rise to the hope that, after improvements, this method would be applicable for the aromatization and possibly for the elucidation of structure of complicated hydro-aromatic compounds, containing side chains and functional groups. One of the most important steps in this direction had to be the lowering of the reaction temperature, in order to avoid pyrolysis. This has thus far been accomplished for tetralin by the addition of catalysts, as described below.

It was found in another investigation[2] that the heterogeneous solid-solid reaction between potassium perchlorate and carbon was strongly catalysed by the presence of various salts, such as potassium carbonate or lithium chloride, i.e. by salts which are able (owing to their basicity) to break down the stable surface oxides which are the primary products of the carbon oxidation[3,4] or by salts which can serve as oxygen carriers[5,6].

We tested at the beginning mainly catalysts of the oxygen-carrier type (chromic oxide, barium dioxide, lead dioxide and some halogenides). These, as well as basic salts, such as potassium carbonate, were found to be totally ineffective. On the other hand, in a third series of tests using oxidation-reduction catalysts of the electron carrying

TABLE I
*Dehydrogenation of tetralin by potassium perchlorate in the presence of various catalysts*

| *Exp. No.* | *Catalyst* | *°C* | *Hours* | *Yield %* $A^{(a)}$ | $B^{(b)}$ |
|---|---|---|---|---|---|
| 1 | None | 370 | 18 | 94 | 87 |
| 2 | ” | 200 | 50 | 0 | 0 |
| 3 | $FeSO_4$ | 200 | 18 | 61 | 71 |
| 4 | $Fe_2(SO_4)_3$ | 200 | 18 | 80 | 100 |
| 5 | $Fe(NO_3)_3$ | 208 | 18 | $15^{(c)}$ | |
| 6 | $FeCl_3$ | $180^{(d)}$ | 18 | $20^{(c)}$ | |
| 7 | $VOSO_4$ | 200 | 30 | 47 | 54 |
| 8 | $Ce(NH_4)(SO_4)_2$ | 200 | 30 | $15^{(c)}$ | |
| 9 | $TiCl_3$ | 200 | 30 | $10^{(c)}$ | |

(a) Calculation based on the initial amount of the tetralin.
(b) Calculated on basis of the potassium perchlorate which decomposed (determined as chloride).
(c) Naphthalene isolated as the picrate, the yield of which, in the presence of tetralin, was not quantitative.
(d) No experiments could be carried out at higher temperatures, as the reaction tubes exploded.

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type, some surprisingly good results were achieved. The results of these experiments are summarised in Table I. All experiments were carried out in sealed heavy-walled pyrex tubes heated in a tubular furnace behind a steel protecting plate, to avoid the danger of explosions which occurred on accidental overheating or owing to faulty reaction tubes. One and a half moles of potassium perchlorate was used for each mole of tetralin and 5% of the weight of the perchlorate was taken of each catalyst. Both perchlorate and catalyst were ground to pass a 200 mesh sieve and mixed intimately by grinding together in a mortar before filling of the reaction tubes. For each experiment 0.9—1.1 g of tetralin were used.

A reaction scheme analogous to some biological oxidation-reduction systems, e.g. cytochromes[7], would seem to be consistent with the facts.

The fact that ferric sulphate (exp. No. 4) gave better results than ferrous sulphate (No. 3) seems to support the view that the ferric ions are the primary oxidants in the reaction. In the absence of a substrate, or even if the substrate is present but is not oxidized in the conditions of the reaction, the ferrous salt itself does not undergo oxidation (e.g. from a mixture of potassium perchlorate, ferrous sulphate and cyclohexane, after heating for 13 hours at 200°C, all the constituents of the mixture, including the ferrous sulphate, were recovered practically unchanged). Two other points are difficult to explain: a) the strong dependence of catalytic efficiency of various ferric salts on the *anion* (experiments No. 4, 5 and 6), and b) the absolute inefficiency of various typical electron-transfer catalysts, such as manganous, cupric and cobaltous salts, which were also tested.

The total pressure in the reaction tube has a very strong influence on the reaction. In the closed tubes the total pressure was about 3 atms. at the beginning of the reaction and it rose, mainly owing to the formation of water, up to 10—15 atms. We attempted to carry out the reaction at reduced pressures in the following apparatus: A pyrex glass tube (heated in an electric furnace) contained either a mixture of potassium perchlorate and the catalyst, diluted with pieces of glass, or the same mixture precipitated from hot concentrated solutions on pumice as carrier. The upper end of the tube was connected by means of ground glass joints to an evaporating vessel, which could be heated to 100°C and contained the tetralin, and the lower end of the tube was connected to a collecting tube held at 0°C. After evacuation by a rotary oil-pump, the apparatus was cut off from the pump; a pressure of 10—12 mm was established in the evaporator and about 0.5—1.0 mm in the collecting tube, causing tetralin to distil slowly (0.2—0.3 ml/hour) through the reaction tube. No trace of naphthalene could be found in the distillate, even at reaction temperatures up to 250°C. In contrast, no experiments could be carried out in closed tubes above 210—220°C, as the tubes exploded after a few minutes. The total failure of the reaction in the "distillation" method could hardly be ascribed to insufficient contact time. Perhaps the concentration of the adsorbed molecules is too small; it is possible that two or more tetralin molecules have to be present at each active spot of the perchlorate-catalyst mixture in order to make the reaction possible.

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# PREPARATION OF SOME POLYMERIC PEPTIDES

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In previous publications[1] it was shown that the acylchloride hydrochlorides of amino acids and peptides undergo polycondensation, yielding polymeric polypeptides. In the present communication, this method is extended to the syntheses of poly-*dl*-phenylalanine, poly-*dl*-valine and poly-glycyl-*dl*-alanine.

Polymerization in all cases was carried out by heating in a high vacuum.

Polymers of phenylalanine and valine had previously been prepared by Astbury and coworkers[2] via the respective *N*-carboxyanhydrides, but no experimental details were given. Preparation of poly-glycyl-*dl*-alanine has not been reported, so far, in the literature.

*Poly*-dl-*phenylalanine hydrochloride*. Preparations consisting of 8 units on the average were obtained. These were insoluble in water, but slightly soluble in glacial acetic acid. They gave a positive biuret reaction on prolonged standing with the reagent and, by boiling with an aqueous ninhydrin solution, particles of the substance acquired a blue coloration.

*Poly*-dl-*valine hydrochloride*. Polymers composed of 18 units on the average were isolated, which were insoluble in water and glacial acetic acid as well as in dilute alkali and acids. Towards ninhydrin and biuret they behaved like the above polymer.

*Poly-glycyl*-dl-*alanine hydrochloride*. The chain length of polymers isolated consisted of 4 units of the dipeptide on the average. These substances, however, were soluble in water and glacial acetic acid, as well as in dilute acids and alkali. They gave positive biuret and ninhydrin reactions.

Since both poly-*dl*-phenylalanine hydrochloride and poly-*dl*-valine hydrochloride are insoluble in the usual solvents, no Van Slyke end-group determination could be carried out, and the chain length of these polymers was calculated from the chlorine content. In the case of poly-glycyl-*dl*-alanine, which is water-soluble, both methods were used and results were in complete agreement.

In a previous publication[1] the synthesis of poly-β-alanine by the acyl chloride method was described. In connection with this, we have studied in some detail the behaviour on standing or heating of methyl and ethyl esters of β-alanine, which in this respect show similarity to the esters of glycine. Fodor has shown, in collaboration with Abderhalden[3], that β-alanine esters on standing yield a crystalline precipitate. On closer investigation it was shown to be a condensation product, consisting of β-alanyl groups in acid-amide linkage[4]. Our experiments confirmed the spontaneous polycondensation of such esters, resulting in the formation of polymers averaging 5 to 7 units,

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which are still water-soluble. Direct heating of the ethyl ester also leads to the production of peptide esters consisting of 7 units on the average.

These polymers may be subjected to further condensation by heating in a high vacuum at 180°, lower temperatures having no apparent effect. Thus a polymeric preparation of an average chain length of 14 units was obtained. This latter substance exhibited a markedly lower solubility in all the usual solvents and end-group assay had, therefore, to be restricted to the determination of the alkoxyl content. In this respect the polymer differs from the poly-β-alanine synthesized by the acyl chloride route[1], which, although of a similar average chain length, is fairly soluble in hot water.

It is interesting to note that, whereas β-alanine esters tend, in contradistinction to α-alanine esters, to undergo polycondensation spontaneously like glycine esters, further thermal polymerization results in the doubling only of the chain length. Glycine esters under similar conditions yield polymers averaging up to 110 units[5].

TABLE I

$$1.\quad NH_2\text{—}R\text{—}COOH \xrightarrow[CH_3COCl]{PCl_5} HCl.NH_2\text{—}R\text{—}COCl$$

$$2.\quad n HCl.NH_2\text{—}R\text{—}COCl \xrightarrow[+(1H_2O)]{-(2n-1)HCl} HCl.H(NH\text{—}R\text{—}CO)_nOH$$

$$R = \left\{ \begin{array}{ll} \begin{array}{c} CH_2\text{—}C_6H_5 \\ | \\ \text{—}CH\text{—} \end{array} & \text{for phenylalanine} \\ \\ \begin{array}{c} CH\text{—}(CH_3)_2 \\ | \\ \text{—}CH\text{—} \end{array} & \text{for valine} \\ \\ CH_2\text{—}CONH\text{—}\underset{\displaystyle CH_3}{\underset{|}{CH}}\text{—} & \text{for glycylalanine} \end{array} \right.$$

TABLE II

$$n\ H_2N\text{—}CH_2\text{—}CH_2\text{—}COOR \longrightarrow H(NH\text{—}CH_2\text{—}CH_2\text{—}CO)_nOR + (n-1)ROH$$

## EXPERIMENTAL

Micro-combustion analyses are by Drs. Weiler and Strauss, Oxford.

dl-*Phenylalanylchloride hydrochloride*[6]. Five g of phenylalanine, reprecipitated from its aqueous solution by absolute ethanol and dried at 110°, was finely ground, driven through a hair sieve and placed in a 250 ml glass-stoppered bottle together with 100 ml of freshly distilled acetylchloride. 7.5 g of phosphorus pentachloride was added and the mixture shaken for 24 hours. The material was finally filtered, washed with a few ml of acetylchloride and several times with dry petroleum ether and dried over phosphorus pentoxide in a vacuum desiccator. Care was taken to exclude moisture in all operations. The yield was 5 g (75%).

*Anal.* Calcd. for $C_9H_{11}ONCl_2$: Cl, 32.2. Found: Cl, 29.8.

*Polymerization of* dl-*phenylalanylchloride hydrochloride in high vacuum.* Two g of *dl*-phenylalanylchloride hydrochloride was placed in the reaction vessel of a high vacuum apparatus and heated at 140—145° for five hours. The yellowish polymer obtained was heated under reflux with 25 ml of absolute ethanol for 15 minutes and then filtered and dried. It was insoluble in water, but slightly soluble in glacial acetic acid. On prolonged contact with the biuret reagent it gave a positive reaction. Boiling with an aqueous solution of ninhydrin resulted in the blue coloration of the particles of the substance.

*Anal.* Calcd. for $C_{72}H_{75}O_9N_8Cl_2$ ($n$ 8): C, 70.1; H, 6.1; N, 9.1; Cl, 2.9. Found: C, 69.6; H, 6.0; N, 9.2; Cl, 3.0.

dl-*Valylchloride hydrochloride.* Seven g of *dl*-valine, suspended in 140 ml of freshly distilled acetylchloride, was shaken with 10 g of phosphorus pentachloride for three hours, at room temperature. After that another 3 g of phosphorus pentachloride was added and shaking resumed for 3 hours more. Filtration, washing and drying were then carried out as usual and 5.5 g (53%) of the substance was obtained.

*Anal.* Calcd. for $C_5H_{11}ONCl_2$: Cl, 41.2. Found: Cl, 40.0.

*Polymerization of* dl-*valylchloride hydrochloride in high vacuum.* Three g of *dl*-valylchloride hydrochloride was heated in high vacuum at 155° for 5 hours. The resulting white polymer was purified by boiling under reflux with water for 30 minutes and washing with ethanol and ether. It was insoluble in water or glacial acetic acid, as well as in dilute acids and alkali. A positive biuret reaction was, therefore, only obtained after prolonged contact with the reagent. Particles of the substance were coloured blue on boiling with an aqueous ninhydrin solution.

*Anal.* Calcd. for $C_{90}H_{165}O_{19}N_{18}Cl_2$ ($n$=18): C, 58.7; H, 9.0; N, 13.7; Cl, 1.9. Found: C, 58.6; H, 9.0; N, 13.7; Cl, 1.9.

*Glycyl*-dl-*alanylchloride hydrochloride.* 9.3 g of the dipeptide, suspended in 150 ml of freshly distilled acetylchloride, was shaken with 15.6 g of phosphorus pentachloride for 20 minutes in an ice-bath and then for 16 hours at room temperature. After the usual treatment, 10 g (78%) of the substance was obtained.

*Anal.* Calcd. for $C_5H_{10}O_2N_2Cl_2$: Cl, 35.2. Found: Cl, 31.2.

*Polymerization of glycyl*-dl-*alanylchloride hydrochloride in high vacuum.* Two g of glycyl-*dl*-alanylchloride hydrochloride was heated in high vacuum at 130° for 2½ hours. The resulting yellowish polymer was purified by dissolving in ethanol and reprecipitation with dry ether. It was soluble in water and glacial acetic acid and gave positive ninhydrin and biuret reactions.

*Anal.* Calcd. for $C_{20}H_{35}O_9N_8Cl$ ($n$=4): C, 42.3; H, 6.2; N, 19.7; Cl, 6.3; Van Slyke N, 2.5. Found: C, 42.0; H, 6.2; N, 17.9; Cl, 6.1; Van Slyke N, 2.9.

*β-Alanine ethyl ester.* The aqueous solution of β-alanine ethyl ester hydrochloride was treated with sodium hydroxide and potassium carbonate in the presence of ether, according to the usual method of E. Fischer[7], and the free ester was finally obtained after distillation in vacuum.

*Polymer isolated from pure ethyl ester.* The ester was kept in a glass-stoppered Erlenmeyer flask for 6 days at room temperature. During that time a white precipitate formed. This was filtered off and washed several times with ether in order to remove monomeric ester. The substance was soluble in water, partially soluble in ethanol, but insoluble in ether. The crude material was of an average chain length of 5—6 units, according to Van Slyke amino nitrogen (Calcd. for $n = 5$—$6$: N, 3.0—3.6. Found: N, 3.3). The fraction insoluble in ethanol was composed of 9 units on the average (Calcd. for $n = 9$: Van Slyke N, 2.0. Found: Van Slyke N, 2.1). Both ninhydrin and biuret reactions were positive.

*Polymer isolated from pure methyl ester.* This ester, prepared similarly, was kept in a refrigerator for 6 days and the precipitate which formed was filtered off and washed with ethanol and ether. According to Van Slyke determinations, this polymer averaged 5 units. Solubility, etc., did not differ from the above polymer.

*Anal.* Calcd. for $C_{16}H_{29}O_6N_5$ ($n = 5$): C, 49.5; H, 7.5; N, 18.1; Van Slyke N, 3.6. Found: C, 47.9; H, 7.5; N, 17.5; Van Slyke N, 3.6.

*Polymer produced by direct heating of β-alanine ethyl ester.* Two g of the ester was heated in a test tube over an open flame, whereby an amorphous white mass was produced. This was triturated with ether and filtered off. The substance was composed of 7 units on the average and behaved like the polymers discussed above.

*Anal.* Calcd. for $C_{23}H_{41}O_8N_7$ ($n = 7$): C, 50.7; H, 7.5; N, 18.0; Van Slyke N, 2.6. Found: C. 49,2; H, 7.5; N, 17.3; Van Slyke N, 2.7.

*Polymerization of β-alanine ethyl ester in ethereal solution.* Four g of β-alanine ethyl ester, dissolved in 15 ml of dry ether, was kept in an ice-box for seven days. The precipitate formed was filtered off and dried. This substance represented a polymer averaging 5—6 units (Calcd. Van Slyke N, 3.0—3.6. Found: Van Slyke N, 3.2).

*Polymerization of β-alanine ethyl ester in high vacuum at* 140°. The latter polymer (averaging 5—6 units) was heated in high vacuum at 140° for 3 hours. Van Slyke determinations then indicated an average chain length of 7 units (Calcd. ($n = 7$): Van Slyke N, 2.6. Found: Van Slyke N, 2.8).

*Polymerization of β-alanine ethyl ester in high vacuum at* 180°. The polypeptide ($n = 7$) was heated in high vacuum at 180° for an additional 3 hours. The resulting substance was almost insoluble in all usual solvents including water, glacial acetic acid and dimethylformamide. On being boiled with an aqueous ninhydrin solution, particles of the substance acquired a blue coloration. The biuret reaction was positive when using a suspension of the substance which had been in contact with dilute alkali for several hours. According to end-group determinations (ethoxyl content) this polymer averaged 14 units.

*Anal.* Calcd. for $C_{44}H_{76}O_{15}N_{14}$ ($n = 14$): C, 50.7; H, 7.3; N, 18.8; ethoxyl, 4.3. Found: C, 50.6; H, 7.3; N, 17.9; ethoxyl, 4.5.

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# A RAPIDLY DEVELOPING AND RAPIDLY WANING EFFECT OF TRIIODO-THYRONINE ON THE CARBOHYDRATE METABOLISM OF THE RAT DIAPHRAGM

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In a previous study[1,2,3] we found that the diaphragm of rats which had received intravenous injections of 20 to 30 μg of thyroxine before excision, showed increased glycogen synthesis and glucose utilization for a short time thereafter if incubated in homologous rat serum but not in Krebs-Ringer phosphate. An equimolecular quantity of thyronine had no effect. Large doses of thyroxine led to decreased glycogen synthesis and frequently to glycogenolysis, and had an irregular effect on glucose utilization.

The addition of thyroxine *in vitro* in a quantity of 2 μg to diaphragms incubated in rat serum, but not in Krebs-Ringer phosphate, increases glycogen synthesis and glucose utilization under certain conditions. Higher doses do not yield the same effect. Thyronine, diiodothyrosine and potassium iodide are without effect. On the basis of these experiments, the hypothesis was formulated that in addition to its slowly developing and gradually waning activity which is measured by a rise in basal metabolism, thyroxine has also a rapidly developing and rapidly waning effect, which may be measured by a rise in glycogen synthesis and glucose utilization by the diaphragm muscle.

It was shown last year that 3,5,3′-*L*-triiodothyronine is a normal constituent of human plasma[4]. This compound has now been isolated in small amounts from fresh ox thyroid and has been shown to be identical with synthetic triiodothyronine.

Triiodothyronine has been reported to be 3—4 times as effective as thyroxine in preventing goiter development in thiouracil treated rats. Also in the treatment of 2 mixoedematous patients it appeared to be effective at doses lower than those required with thyroxine administration. These experiments were confirmed by many other investigators. It may very well be possible that this compound is responsible for the peripheral action of the thyroid gland. It was of interest to investigate if after intravenous injection of triiodothyronine we shall also observe the rapidly developing and rapidly waning effect which may be measured by a rise in glycogen synthesis and glucose utilization by the diaphragm muscle, as we found out for thyroxine.

## RESULTS

The intravenously injected triiodothyronine has the following effects: a beginning reaction on glycogen synthesis of the diaphragm at 2 μg, a definite reaction at 5 μg, a maximal effect at 10 μg and a decreasing one at 20 — 30 μg; and a steady effect on glucose utilization. Simultaneous experiments with intravenous injections of thyroxine showed a questionable effect at 5 μg, a definite reaction at 10 μg, and a maximal effect at 20 μg. In previous experiments the effect of thyroxine began at 5 μg, was more certain at 10 μg,

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and reached a maximum at 20 — 30 μg. The effect of triiodothyronine — according to these experiments — should be about three times as great as that of thyroxine (Table I).

TABLE I

*Glycogen synthesis and glucose utilization by rat diaphragms after injection of varying amounts of triiodothyronine*

| *Dose of triiodothyronine μg/100 gm body weight* | *No. of rats* | *Glycogen synthesis mg/100 mg diaphr.* | *Glycogen difference mg/100mg diaphr.* | *Glucose utilization mg/100mg diaphr.* | *Gluc. utilization difference mg/100mg diaphr.* |
|---|---|---|---|---|---|
| 0 | 56 | 0.068 ± 0.0035 | | 0.72±0.01 | |
| 1 | 4 | 0.069 | 0 | 0.68 | |
| 2 | 19 | 0.095 | 0.027±0.005 p=0.01 | 0.90 | 0.18±0.045 p=0.01 |
| 5 | 23 | 0.108 | 0.040±0.007 p=0.01 | 0.81 | 0.09±0.028 p<0.01 |
| 10 | 23 | 0.131 | 0.063±0.007 p=0.01 | 0.85 | 0.13±0.036 p<0.01 |
| 20—30 | 27 | 0.088 | 0.020±0.007 p=0.01 | 0.86 | 0'14±0.036 p<0.01 |

Diiodothyronine was not effective in corresponding or higher concentrations. Dibromothyronine was of weak and questionable effect (Table II).

TABLE II

*Glycogen synthesis and glucose utilization by diaphragms of normal rats after injection of dibromothyronine* (20—40 μg) *and diiodothyronine* (20—50 μg)

| *Substance injected* | *No. of rats* | *Glycogen synthesis mg/100 mg diaphragm* | *Glycogen difference mg/100 mg diaphragm* | *Glucose utilization mg/100 mg diaphragm* | *Glucose utilization difference mg/100 mg diaphragm* |
|---|---|---|---|---|---|
| Saline | 31 | 0.068 | | 0.72 | |
| Dibromothyronine | 25 | 0.078 | 0.010±0.0045 *p*=0.04 | 0.80 | 0.08 ±0.035 *p*=0.02 |
| Diiodothyronine | 6 | 0.057 | —0.011±0.011 *p*=0.30 | 0.75 | 0.03 ±0.03 *p*=0.30 |

The start of the reaction of triiodothyronine was not discernible at 15—30 mins. after intravenous injections. In the average of five experiments the glycogen synthesis after triiodothyronine administration was 0.077 mg/100 mg diaphragm. The control also gave 0.077 mg/100 mg diaphragm. The corresponding glucose utilization was 0.93 mg compared with 0.83 mg/100 mg diaphragm. The difference is not significant. After 45 mins., however, the difference became clear. (Glycogen synthesis was 0.093 as compared with 0.070 mg/100 mg diaphragm, and glucose utilization was 1.16 mg as compared with 0.81 mg/100 mg diaphragm). If triiodothyronine was added to the medium *in vitro*, an effect on glycogen synthesis was noted with the addition of 1 μg. The effect was clearer yet with from 2 — 5 μg. This effect is certainly not stronger than the corresponding effect of thyroxine[2] (Table III).

## DISCUSSION

The following results should be noted: If added intravenously, triiodothyronine has an effect three times as great as thyroxine on glycogen synthesis from glucose, and on glucose utilization of the rat diaphragm, when homologous serum is used as the medium.

TABLE III

*Glycogen synthesis and glucose utilization by rat diaphragms incubated in serum with varying amounts of triiodothyronine.*

| *Concentration of triiodothyronine μg/ml medium* | *No. of rats* | *Glycogen synthesis mg/100 mg diaphragm* | *Glycogen difference mg/100 mg diaphragm* | *Glucose utilization mg/100 mg diaphragm* | *Glucose utilization difference mg/100 mg diaphragm* |
|---|---|---|---|---|---|
| 0 | 46 | 0.103 | | 0.83 | |
| 0.1 | 18 | 0.110 | 0.007 ±0.016<br>p=0.60 | 0.79<br>0.79 | —0.04 ±0.028<br>p=0.20 |
| 0.2—0.5 | 16 | 0.110 | 0.007 ±0.010<br>p=0.40 | 0.78<br>0.78 | —0.05 ±0.033<br>p=0.18 |
| 1 | 12 | 0.130 | 0.027 ±0.012<br>p=0.05 | 0.94 | 0.11 ±0.036<br>p=0.02 |
| 2—5 | 14 | 0.144 | 0.041 ±0.011<br>p=0.01 | 0.82 | 0.01 |
| 10 | 17 | 0.121 | 0.018 ±0.012<br>p=0.035 | 1.07 | 0.24 ±0.070<br>p=0.01 |

Diiodothyronine, dibromothyronine and thyronine have either no effect at all, or a very uncertain and weak one.

The experiments show that triiodothyronine is the only thyroid hormone which has a stronger effect than thyroxine on glycogen synthesis and glucose utilization of rat diaphragm. Hence the known effect on basal metabolism is parallel to the described effect on carbohydrate metabolism of the diaphragm. It is well known that the effect on basal metabolism starts and disappears slowly, in contrast to the described effect on the carbohydrate metabolism which starts within a short time and disappears after a few hours. These experiments indicate strongly that the active hormones of the thyroid gland show, in addition to the slowly beginning and disappearing effect on basal metabolism, still another reaction which starts quickly and passes quickly. This rapid effect can be shown by measuring the glycogen synthesis and glucose utilization of the rat diaphragm. This reaction is obtained by doses which can still be called physiological. The effect on carbohydrate metabolism on the diaphragm could be shown 45 min. after the addition of triiodothyronine, i.e. not sooner than in previous experiments with thyroxine[2].

There was no certain difference to be seen between the effect *in vitro* of thyroxine and triiodothyronine.

## CONCLUSION

Intravenously administered triiodothyronine has an effect about three times as great as thyroxine on the carbohydrate metabolism of rat diaphragm. Contrary to the known effect on basal metabolism, it begins very soon and disappears after a few hours.

Diiodothyronine, dibromothyronine and thyronine are almost or completely ineffective. With regard to time, this effect of triiodothyronine appears no sooner than the effect of thyroxine.

Triiodothyronine added *in vitro* to the rat diaphragm medium has no stronger effect than thyroxine on the carbohydrate metabolism of the diaphragm.

Our best thanks to Dr. R. Pitt-Rivers for her generous gift of triiodothyronine.

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# PECTIN FROM SUNFLOWER HEADS

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The purpose of this work was to determine:

a) whether sunflower is a suitable raw material for pectin production,
b) optimal conditions for extraction of pectin from sunflower heads.

## PECTIN CONTENTS IN SUNFLOWER HEADS

Sunflower heads were used after removal of the seeds, as this is the part richest in pectin[1].

The total pectin content was determined by repeated hot acid extractions[2], till the extract gave no more precipitation of pectin with alcohol. All the extractions were mixed and the percent of pectin was determined by optical rotation[3]. Four samples tested gave the following results in percent on dry basis: 20.6, 25.3, 21.4, 25.7. Moisture of sunflower heads, seed-free: 82.0%.

## DETERMINATION OF OPTIMAL EXTRACTION CONDITIONS

Optimal conditions for the extraction of pectin from sunflower heads, as far as industry is concerned, are those yielding maximum jelly units out of a given weight of raw material. Acid extraction was chosen as the generally used and industrially suitable procedure.

The efficiency of extraction depends chiefly on three factors: a) temperature, b) pH, c) time of extraction. A change in these factors induces a change in the amount of insoluble pectin constituents brought into solution; on the other hand, these factors chiefly determine the extent of degradation and de-esterification of pectins.

TABLE I

*Extractions made at different pH's at a temperature of* 100 °C *and a constant extraction time of* 30 *minutes*

| *Initial pH* | *Final pH* | *% of total pectin on fresh fruit* | *% Ca-precipitable pectin* | *Jelly grade* | *Total jelly units* |
|---|---|---|---|---|---|
| 3.0 | 3.75 | 0.43 | 50.0 | | |
| 2.8 | 3.60 | 0.50 | 55.6 | | |
| 2.6 | 3.30 | 0.67 | 54.5 | | |
| 2.4 | 3.10 | 0.78 | 54.5 | | |
| 2.2 | 2.85 | 0.95 | 51.6 | 140 | 533 |
| 2.0 | 2.45 | 1.14 | 47.3 | 200 | 914 |
| 1.8 | 2.25 | 1.43 | 45.4 | 194 | 1120 |
| 1.6 | 2.10 | 1.59 | 49.0 | 185 | 1178 |
| 1.4 | 1.85 | 1.62 | 37.2 | 190 | 1197 |
| 1.2 | 1.60 | 2.24 | 26.2 | 117 | 1048 |

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*a) Temperature*

It has been shown that extractions above the boiling point (under pressure) were unsuccessful, while extractions at 40 °C and below require a longer treatment resulting at times in excessive de-esterification[4]. Workable temperature is within the range 40°—100 °C. Extraction at 100 °C was used for our experiments.

*b) pH*

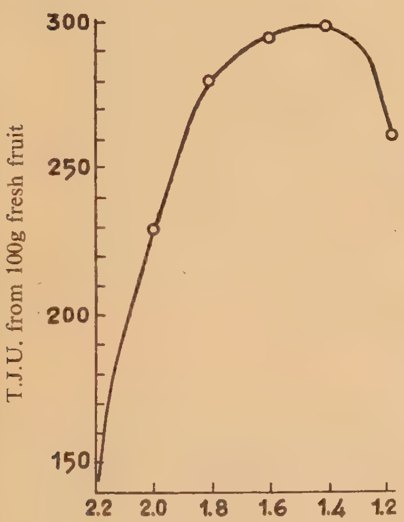


Figure 1

*Optimal initial pH for the extraction, according to the highest total jelly units obtained, was* 1.5.

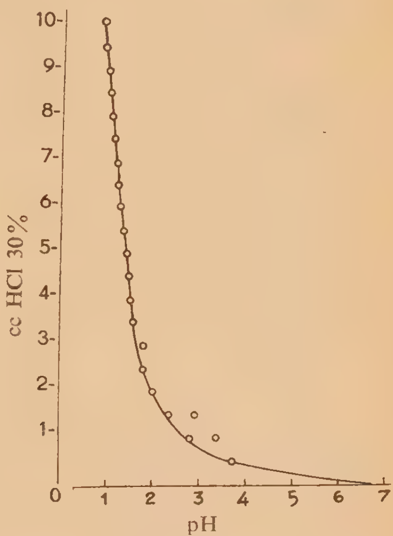


Figure 2

*c) Time of extraction*

At 100 °C and pH 1.5, extractions were carried out with different lengths of time. The optimal yield in jelly units was obtained with a 45 minutes extraction time (Table II).

TABLE II

| *Time of extraction* | *% Pectin on fresh fruit* | *Jelly grade* | *Total jelly units* |
|---|---|---|---|
| 15 | 1.37 | 185 | 1015 |
| 30 | 1.60 | 192 | 1227 |
| 45 | 1.65 | 190 | 1248 |
| 60 | 1.72 | 156 | 1073 |
| 75 | 1.75 | 118 | 822 |

METHODS

*Extraction*

400 g fresh sunflower seed-free heads were ground, treated with boiling water for 10 minutes in order to inactivate the enzymes[5], and then repeatedly washed in cold water. The washed material was added to 1500 cc water in a 3 litre open stainless steel pan.

The pH was adjusted with commercial HCl as shown in Figure 2, and checked by Beckman pH-meter. The time of extraction was measured after the mixture's original weight was restored with water. The extract was filtered free from pulp and cooled to prevent additional degradation.

*Percent of total pectin*

Oxalic acid was added to the cooled extract, in order to solubilize low-ester pectins by precipitating $Ca^{++}$. Then the percent of pectin was determined by optical rotation[3].

*Percent of Ca-precipitable low-ester pectin (methoxyl 4—7%)*

A procedure for precipitation of pectinic acid with a sufficient percent of free carboxyl groups with alkaline earth metals was described by Wallerstein[6].

To a portion of the extract, $CaCl_2$ solution was added. After vigorous stirring, the Ca-pectinate was separated by filtration. The filtrate was tested with additional $CaCl_2$ for low-ester pectin and, if the latter was not present, the percent of pectin in the filtrate was determined by optical rotation[3]. The percent thus determined was subtracted from the percent of the total pectin found in the extract, and thereby the percent of low-ester pectin was obtained.

*Total jelly units*

Part of the extract was used for pectin precipitation. Ethanol 96% was added to the filtrate under vigorous stirring, until concentration over 50% was reached. The mixture was stirred for a short time, the precipitate was filtered, shredded and dried at 56°—75 in an electric oven. The dry pectin was ground and used for preparing the jellies. The jellies were cooked on a balance and the sag measured according to Cox and Higby[7] 160 g of water were boiled in an open aluminium pan. Citric acid was added in an amount previously determined, in order to attain pH 3.0.

From 3 to 5 g pectin were added (depending on the expected grade) mixed with part of the 260 g sugar to be used for the cooking. The balance of sugar was added in two portions and the mixture was boiled down to 400 g (Ca 68° Bx). After 1 minute standing at room temperature the jelly was skimmed and poured into the test glasses. The sagometer was locally made, using a micrometer screw accurate to 1/100 millimetre. As test glasses, conical plastic cups were used, 80 millimetre high. A calibrated curve was established, using a commercial 200° citrus pectin which was accepted as such. Jellies of various strength were prepared with the same pectin. The curve gave jelly units versus the percent of sag. Using this curve[5], the total jelly units of the jellies were determined.

JELLY GRADE

The total jelly units divided by the grams of pectin used in the jelly cooking, yielded the grade of the pectin sample.

## CONCLUSIONS

Sunflower heads free from seeds are a comparatively rich raw material for pectin production, as they contain 20—25% thereof on dry basis.

Optimum extraction conditions were found to be 45 minutes extraction at 100°C in an extraction medium of initial pH 1.5 or final pH 1.75. At optimum extraction conditions, 100 g of raw material yield 8.9 g pectin 190° or 1690 jelly units (See Table I).

After 30′ boiling, an appreciable shift of pH of the extract takes place. At initial pH less than 1.4, there is a considerable fall of the total jelly units extracted.

Below pH 1.4, there appear strong degradation conditions, not accounted for by demethoxylation (See Table I — % of Ca-precipitable pectin).

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# THE STRUCTURAL PATTERN OF PALESTINE (ISRAEL AND CIS-JORDAN)

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We distinguish three fundamental tectonic elements: folding, warping and fracturing. Folding of late Oligocene — early Miocene age leads to a series of anticlines and synclines of prevalent NE or NNE trend. From a morphological viewpoint the best preserved region of foldstructure is found in the Negev. In this arid region a classical Jura fold morphology with trellis patterns, cluses, ruzes and erosion-cirques becomes evident. Perfect anticlines are scarcely disturbed by displacements of the small transcurrent adjustment faults of the Northern Negev. Nor are they too much influenced by the few larger strike faults of the Central Negev. The strike faults are likely to be connected with the asymmetric overturning — noticed in many Negev folds. The chains of folds culminate in an anticlinorial way in the upwarps of the Meneiaie in the South (incomplete because of Graben faulting), of Raman in the Centre and of Kurnub in the North [1,2].

Judea is similarly rich in folds. Apart from some smaller anticlines in the Judean Desert, however, Judea differs by a more "resequent" (Davis) fold morphology and the absence of a Jura-fold relief. This may find its reason in the predominantly semi-arid climate as well as in a certain bilateral fold asymmetry directed either to the downwarped coastal plain of the West or to the equally downwarped Judean Desert of the East. Asymmetry and warping are apparently causally connected. The Judean double-sided asymmetry does not tally with the conception of a unilateral pressure towards the Arabian Shield. This is still more evident from the distinct NW asymmetry of the major anticlines of Western Transjordan which display in this way a structural counterpart to the SE overturned folds of the Northern Negev and Judean Desert. Pleistocene Graben faults have frequently and in an angular manner cut these Upper Tertiary folds.

This phenomenon does not exclude, however, the existence of pre-Pleistocene faults assumed to have given origin to many a Neogene depression in Israel and the Levant. Such Neogene tensional ruptures may also be deduced from the frequent occurrences of Mio-Pliocene basalts and the absence of acid lavas.

The influence of Pleistocene Graben tectonics is felt only at the approach of the crests overlooking the Southern Arabian valley or at the very edge of the cliffs of the Dead Sea. Small graben and horsts and still more stepped blocks outlined by many arcuate and *en echelon* (crescentic, sinoidal, rotational) faults, have masked a main border fault on the western side of the Wadi Araba — Dead Sea rift valley. The curved faults with an average throw of 100 m and a maximum of 500 m (Quruntul), continue to model the western slopes of the Jordan Valley up to Northern Palestine. In Samaria they enter

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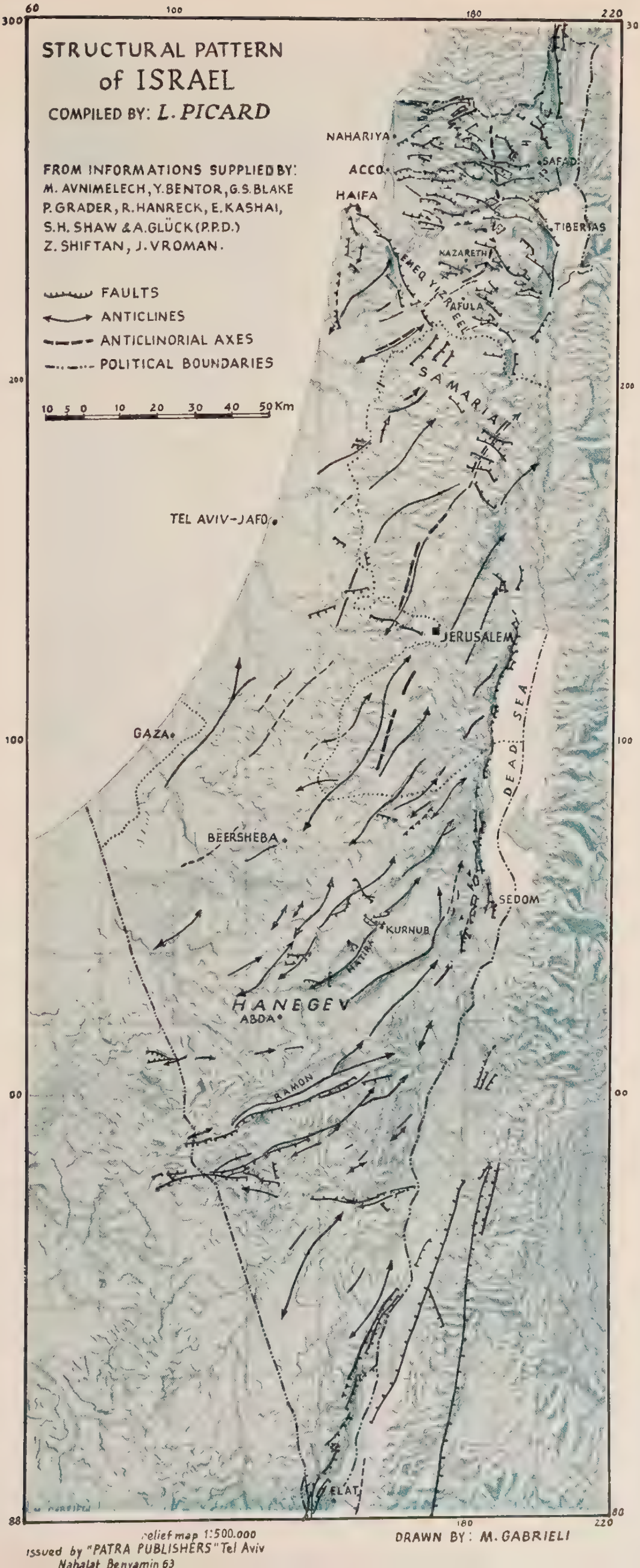

STRUCTURAL PATTERN of ISRAEL
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FAULTS
ANTICLINES
ANTICLINORIAL AXES
POLITICAL BOUNDARIES
10 5 0 10 20 30 40 50 Km
NAHARIYA
ACCO
HAIFA
SAFAD
TIBERIAS
NAZARETH
EMEQ YIZREEL
AFULA
SAMARIA
TEL AVIV-JAFO
JERUSALEM
DEAD SEA
GAZA
BEERSHEBA
SEDOM
KURNUB
HATIRA
HANEGEV
ABDA
RAMON
ELAT
60
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far into the mountain body and in Galilee they become the dominant tectonic feature. Samaria is indeed strongly block-faulted. Tilted blocks appear with the approach of the Gilboa mountains, forming fault angle depressions (Sahl Arraba, Marj Sanur, Bukeia) or intermountain graben (Wadi Fari'a, Beth Dajan). The latter cross the plunging East-Samarian arch — a possible continuation of the Judean upwarp zone. Such an arch — adjoining the synclinorial Megiddo — or Ephraim basin — is also the bearer of the Umm El Fahm anticline, with its "Judean" West, or NW, asymmetry. The Carmel is an uplift, the folding and warping elements of which are not yet analyzed in a satisfactory manner. Thus, Samaria — Ephraim—Carmel take up a transitional position between the fold decorated Negev and Southern Judea and the dense fault pattern of Galilee.

In Galilee, the outlining of an anticline is hardly possible; the folds are completely split up in fault blocks and so is the postulated Galilean upwarp. Eastern Galilee, from Mount Gilboa to the Tiberias Lake — the region of an uplifted Neogene basin — is distinguished by an impressive grouping of tilted blocks, each of their fold scarps facing North (or rather NE). Sheet basalts of Upper Pliocene age on the back slope have transformed the tilted blocks into typical louder-backed mountains. The insignificant ravines of the gentle slope join consequently the major wadi, which follows parallel to, and drains the fault-angle depression. Only in one instance (Wadi Hammam) has the wadi of the back-slope been cut by a deep gorge traversing the crest line of the scarp and entering the adjoining fault depression (Migdal). In this area, as elsewhere in East Galilee, the NW—SE directed fault scarps turn crescentically to the south and form the western border of the Lake and Graben of Tiberias, where the hot springs rise to the surface. The contemporaneity and structural unity of Pleistocene Graben tectonics and tilted block formation is here clearly demonstrated.

In the area north and north-east of Safad a new set of semi-circular faults embracing tilted blocks appears again with N or NE-facing scarps. This region, which we attach to a new upwarp or uplifted zone (Hunin uplift), was completely destroyed by the breakdown of the Upper Jordan — or Huleh — Graben. Youthful gorges conditioned by the faults and running parallel to them, enter the Huleh Graben. Here, the existing major border fault dies out in a crescent-shaped ending (Ayeleth Hashahar).

The much broader Western Galilee, the district west of the watershed, is crossed principally by E—W fractures. They form a western group of tilted blocks, extending from the Lebanon down to the Kishon plain. Contrary to the eastern group, their fault scarp faces south (rarely SE). Moreover, a few transversal graben of similar E—W trend alternate with the tilted blocks. At the western mountain border (Wadi Halazun), they seem — according to gravity measurements — to continue below the coastal plain. They may still be found in the submarine depressions (see map) of the Acre—Haifa Bay and the Lebanon. In the centre of the mountains, meeting the western fault system, the big graben-like E—W depression of Battauf appears. Instead of distinct border faults, a set of shorter curved fault lines defines the graben edge. Similarly, the Tabor Graben, another impressive E—W depression, forms the structural "meeting place" between the East and West systems. Great vertical displacements occurred in this watershed region; they are partly responsible for the outcrop of the oldest formations: Albian —Aptian ("A" on the map).

The watershed region has thus proved to be a marked morphological and structural

divide. On this structural "backbone" the fracture systems of East and Western Galilee part (Tabor, Nimrin, Eilabun, Sasa), faults and blocks rotate, the fault-scarps of the tilted blocks face two different directions. Twisting took place along a N—S axis, which may coincide with the crest line of a former Galilean upwarp. Conspicuously placed on this tectonic vertex, we encounter the mountains of Tabor, Hazzur, Heidar, Adathir, Jarmak, surrounded by faults of both systems. These "neutralized" horsts, characteristically enough, have no single fault-scarp face but steep slopes in all directions. The collapse of Galilee, as shown by the crescentic faults of Tiberias, is closely connected with the Pleistocene down-faulting of the Jordan Graben. Tilted block-faulting of East Galilee and Jordan Graben are here one unity. Thus, we feel justified in assuming that the western block-system which forms Western Galilee might be causally connected and contemporaneous with the Pleistocene tafrogenic breakdown of the Mediterranean.

The Pleistocene movement of the faulted blocks of Galilee continues to the Recent. The fault-scarps are splintered by hundreds of *en echelon* earthquake scarplets and earthquake rents. Slickenslide varnishes, fault-breccias, gouges are perfectly preserved. Fault valleys are still in the initial erosional cycle, the main features of the fault blocks being only slightly modified by erosion. The continuous movements of blocks have kept the relief in a youthful stage far superior to that found in the fold-dominated Negev and Southern Judea.

Each section of the country, thus, has a certain morphotectonic individuality: the Negev—a Jura-fold morphology of early maturity, Judea — mature and broad upwarps, Galilee — youthful basin structures, — though in each section we may find the influence and the forms of all the major tectonic phenomena: folding, warping and faulting.

On the whole the fault-lineament of Israel reveals a prevalence of transversal and meridional faults crossing the fold axes. Longitudinal faults running parallel with the strike of the folds play a minor role and are comparatively rare. There exists, therefore, little parallelism between fault and fold directions. This observation disfavours the tendency found in recent literature to trace back the pattern of major and minor folds to movements of faults in the basement, as far as the Late Pliocene—Protopleistocene tafrogeny is concerned.

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# STUDIES ON THE ACTION OF POLYLYSINE ON THE FIBRINOLYTIC REACTION

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Human plasma and plasma of various animal species are known to contain a proenzyme (profibrinolysin, plasminogen) which can be activated to an enzyme (fibrinolysin, plasmin) capable of dissolving fibrin clots and of rendering fibrinogen unclottable by thrombin[1,2,3]. Profibrinolysin is activated to fibrinolysin by streptokinase[1], staphylokinase[4], tissue fibrinolysokinase[5,6] or by chloroform[7]. In a previous communication[8] it was reported that the synthetic water soluble poly-α-amino acids, polylysine, polyornithine and polyarginine, inhibit lysis of human clots induced by menstrual blood, or by human plasma treated with streptococcal suspensions or with chloroform. In the present study an attempt was made to elucidate the site of the inhibitory action of polylysine on the fibrinolytic system, whether on the activation of profibrinolysin to fibrinolysin or on the lysis of the clot by the fibrinolysin.

In addition observations on the effect of polylysine on the clotting of partially lysed fibrinogen by thrombin are presented.

## MATERIALS AND METHODS

Bovine Fraction I (Armour Co.) containing 50% sodium citrate. This fibrinogen preparation was found to contain a small amount of profibrinolysin, as shown by incubation with streptokinase which resulted in slow dissolution of the clot formed by the addition of thrombin.

Human plasma, obtained from venous blood to which 1/9 volume 0.1 M sodium oxalate was added.

Rat blood was obtained by cardiac puncture and rendered incoagulable with 1/9 volume 0.1 M sodium oxalate.

Crude profibrinolysin was prepared from human serum by the method of Ratnoff[9]. Purified bovine fibrinolysin (Batch Rx 164678) was kindly supplied by Dr. E. Loomis from the Research Laboratories of Parke, Davis & Co. This preparation does not contain profibrinolysin activator, as tested by incubation with profibrinolysin and subsequent measurement of fibrinolytic potency.

Streptokinase-streptodornase (Varidase-Lederle) containing 148,000 units of streptokinase per vial.

Fibrinolysin containing oxalated plasma or serum obtained from patients 15 minutes after electroshock. The serum was lacking in profibrinolysin activator, as tested by incubation with profibrinolysin and subsequent measurement of clotting potency.

Bovine thrombin (Upjohn Co.) containing 1000 units per vial.

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Poly-*L*-lysine hydrobromide of average degree of polymerization $n=36$, prepared according to the method of Katchalski, Grossfeld and Frankel[10].

All solutions were made up with barbital saline buffer, pH 7.4 (sodium barbital 8.244 g, 0.1 N HCl 288 ml, potassium oxalate 0.2948 g, NaCl 11.0 g, made up to 2000 ml with distilled water).

Determination of fibrinolytic activity: to mixtures containing Fraction I or plasma, the fibrinolytic enzyme and polylysine, thrombin was added in a final concentration of 5 units per ml mixture; the intervals required for complete disappearance of the clot at 37°C (lysis time) were measured.

Determination of fibrinogenolytic activity: mixtures containing plasma or Fraction I, the fibrinogenolytic enzyme and polylysine were incubated at 37°C; samples were withdrawn after various intervals of time and tested for the presence of clottable fibrinogen by the addition of thrombin (final concentration 5 units per ml mixture). The intervals required for the appearance of a coherent clot were measured.

## EXPERIMENTAL

### I. *Inhibition of the action of fibrinolysin on fibrin by polylysine*

1. Inhibitory action of polylysine on dissolution of clotted Fraction I by purified bovine fibrinolysin.

The data given in Table I show that lysis of clotted Fraction I by purified bovine fibrinolysin was delayed by polylysine in the concentration of 75 μg/ml mixture and higher. As the fibrinolysin preparation did not contain profibrinolysin activator, the delay in clot lysis in the presence of polylysine cannot be due to an inhibitory action of the latter on activation of profibrinolysin present in Fraction I, but is caused by its action on the clot dissolution reaction itself.

2. Inhibitory action of polylysine on dissolution of human plasma clot and of clotted Fraction I by "electroshock fibrinolysin".

TABLE I

*Inhibitory action of polylysine on dissolution of clotted Fraction I by purified bovine fibrinolysin* *

| *Clotted Fraction I mg/ml mixture* | *Purified bovine fibrinolysin μg/ml mixture* | *Poly-L-lysine hydrobromide μg/ml mixture* | *Lysis time at 37°C minutes* ** |
|---|---|---|---|
| 3 | — | — | no lysis |
| 3 | — | 1000 | no lysis |
| 3 | 12.5 | — | 120 |
| 3 | 12.5 | 50 | 180 |
| 3 | 12.5 | 75 | 240 |
| 3 | 12.5 | 150 | no lysis |
| 3 | 12.5 | 300 | no lysis |
| 3 | 12.5 | 600 | no lysis |
| 3 | 12.5 | 1000 | no lysis |

* Reaction mixture: Fraction I, 0.5 ml
purified bovine fibrinolysin, 0.05 ml
poly-*L*-lysine hydrobromide, 0.1 ml
made up to 0.9 ml with saline barbital buffer, pH 7.4
thrombin solution containing 50 units per ml, 0.1 ml.

** Observation of clot lysis was discontinued after 24 hours.

The results presented in Table II show that lysis of clotted plasma and clotted Fraction I by serum obtained from a patient after electroshock was delayed by polylysine. As the "electroshock serum" was lacking in profibrinolysin activator, the delay in clot dissolution by polylysine cannot be explained by an inhibitory action of the polymer on profibrinolysin activation, but is due to its action on the clot lysis reaction itself.

TABLE II

*Inhibitory action of polylysine on dissolution of clotted human plasma or of clotted Fraction I by "electroshock plasma or serum"* *

| *Clotted Fraction I mg/ml mixture* | *Electroshock serum, ml* | *Electroshock plasma, ml* | *Poly-L-lysine hydrobromide μg/ml mixture* | *Lysis time at 37°C, minutes*** |
|---|---|---|---|---|
| — | — | 0.5 | — | 95 |
| — | — | 0.5 | 200 | no lysis |
| 3 | 0.3 | — | — | 180 |
| 3 | 0.3 | — | 200 | no lysis |
| 3 | — | — | — | no lysis |
| 3 | — | — | 200 | no lysis |

* Reaction mixture: a. for "electroshock plasma" experiment:
plasma, 0.5 ml
poly-*L*-lysine hydrobromide, 0.1 ml
made up to 0.9 ml with saline barbital buffer, pH 7.4
thrombin solution (50 units per ml), 0.1 ml.
b. for "electroshock serum" experiment:
Fraction I, 0.5 ml
serum 0.3 ml
poly-*L*-lysine hydrobromide, 0.1 ml
made up to 0.9 ml with saline barbital buffer, pH 7.4
thrombin solution (50 units per ml), 0.1 ml.

** Observation of clot lysis was discontinued after 24 hours.

3. Inhibitory action of polylysine on dissolution of rat plasma clot by streptokinase-activated human plasma and by purified bovine fibrinolysin.

Human plasma activated by streptokinase is known to dissolve rat plasma clot. On the other hand it is known from the literature[11] and confirmed in the experiment described below (Table III) that incubation of rat plasma with streptokinase does not result in fibrinolytic activity. It follows that the lysis of the rat plasma clot by human plasma incubated with streptokinase is not due to rat plasma fibrinolysin but is the result of the action of the human fibrinolysin. The data given in Table III, showing that polylysine delayed lysis of rat plasma clot by streptokinase-activated human plasma, prove therefore that polylysine has an inhibitory action on the dissolution of the clot by the activated fibrinolysin.

In addition the inhibitory action of polylysine on the clot dissolution reaction is clearly shown by the experiment with purified bovine fibrinolysin.

## II. *The action of polylysine on the clotting of partially lysed fibrinogen by thrombin*

During our investigation of a possible action of polylysine on fibrinogenolysis it was found that Fraction I, lysed by bovine fibrinolysin to the extent where thrombin was no longer able to induce clotting, still could be clotted by thrombin in the presence of polylysine. This is demonstrated by the following representative experiment (Table

TABLE III

*Inhibitory action of polylysine on dissolution of rat plasma clot by streptokinase-activated human plasma and by purified bovine fibrinolysin* *

| *Clotted rat plasma ml* | *Streptokinase units per ml mixture* | *Streptokinase-activated human plasma*** *ml* | *Purified bovine fibrinolysin µg/ml mixture* | *Poly-L-lysine hydrobromide µg/ml mixture* | *Lysis time at 37°C, minutes**** |
|---|---|---|---|---|---|
| 0.5 | — | — | — | — | no lysis |
| 0.5 | 10 | — | — | — | no lysis |
| 0.5 | — | — | — | 500 | no lysis |
| 0.5 | 10 | — | — | 500 | no lysis |
| 0.5 | — | 0.1 | — | — | 18 |
| 0.5 | — | 0.1 | — | 500 | 95 |
| 0.5 | — | — | 25 | — | 90 |
| 0.5 | — | — | 25 | 100 | no lysis |

* Reaction mixture: oxalated rat plasma, 0.5 ml
streptokinase-activated human plasma or purified bovine fibrinolysin, 0.1 ml
poly-*L*-lysine hydrobromide, 0.1 ml
saline barbital buffer was added to the final volume of 0.9 ml
thrombin solution containing 50 units per ml, 0.1 ml.

** To oxalated human plasma 1/19 volume of streptokinase solution was added to a final concentration of 100 units per ml mixture; the mixture was incubated for 10 minutes at 37°C. The incubation mixture was shown to have fibrinogenolytic activity by the demonstration of lack of clot formation on the addition of thrombin.

*** Observation of clot lysis was discontinued after 24 hours.

IV). Bovine Fraction I was incubated with purified bovine fibrinolysin. After various periods of incubation, indicated in Table IV, aliquots were withdrawn and thrombin was added with or without polylysine. The clotting times were measured at 37°C and recorded in the table.

TABLE IV

*Action of polylysine on the clotting of partially lysed fibrinogen by thrombin*

| *Samples of fibrinogenolytic mixture*, ml* | *Poly-L-lysine hydrobromide, µg/ml mixture* | *Thrombin units per ml mixture* | *Periods of incubation of fibrinogenolytic mixture minutes* | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 5 | 10 | 20 | 25 | 30 | 35 | 40 | 50 | 60 | 70 |
| | | | *clotting times, seconds* | | | | | | | | | | |
| 0.5 | — | 5 | 30 | 50 | 80 | 150 | — | — | — | — | — | — | — |
| 0.5 | 40 | 5 | 17 | 20 | 25 | 40 | 50 | 65 | 75 | 80 | 90 | 140 | 190 |

* Fibrinogenolytic reaction mixture:
Fraction I (1 g/100 ml), 4.3 parts
purified bovine fibrinolysin (5 mg/ml), 0.2 parts
made up to 5 parts with saline barbital buffer.

In addition the data in Table IV (incubation period 0) confirm the observation reported elsewhere that polylysine accelerates the clotting of unlysed fibrinogen by thrombin[12]. Repeated experiments showed that after complete lysis by bovine fibrinolysin of fibrin clots obtained by the clotting of Fraction I with thrombin, the addition of polylysine and thrombin did not result in the appearance of a visible clot.

## DISCUSSION

The experimental data obtained in this study show that polylysine has an inhibitory action on the lysis of human, bovine and rat fibrin clots by active fibrinolysin. Polylysine inhibits clot lysis by fibrinolytic enzymes obtained from various sources: purified bovine fibrinolysin, fibrinolysin present in human plasma after electroshock treatment, and human plasma fibrinolysin activated by streptokinase.

Bidwell[13] demonstrated that the fibrinolytic enzymes present in cadaver plasma and in plasma after physical stress are different from that evolved in streptokinase-activated human plasma. While the latter digests fibrinogen as well as fibrin and is inhibited by plasma antifibrinolysin, the former digest fibrin only and are not affected by antifibrinolysin. We have found that the fibrinolytic enzyme of electroshock plasma resembles the cadaver and stress lysins in its inability to digest fibrinogen and its resistance to inactivation by antifibrinolysin. Since the experiments reported here demonstrate that polylysine has an inhibitory action on clot dissolution by streptokinase-activated human plasma as well as electroshock plasma lysin, it follows that the basic polypeptide inhibits both types of fibrinolysin.

It has not been possible to decide whether polylysine also inhibits the reaction of the activation of profibrinolysin to fibrinolysin; pertinent experiments gave erratic results.

During our investigation of a possible action of polylysine on lysis of fibrinogen it was observed that polylysine is able to induce clotting when added to mixtures of partially lysed fibrinogen and thrombin, which do not clot in the absence of the basic polyamino acid. It was further observed that on incubating fibrinogen with fibrinolysin in the presence of polylysine, fibrinogen remained detectable by thrombin for a longer time and the clotting times were shorter than without polylysine. From these data no final conclusion can be drawn as to a fibrinogenolysis-inhibitory action of polylysine, since the results may be due either to the clot promoting action of polylysine in the fibrinogenolytic system, or to inhibition of fibrinogenolysis.

It has been found that polylysine is able to induce a clot in mixtures of thrombin with small amounts of fibrinogen, such as are not clottable in the absence of the polyamino acid[12]. The clot promoting activity of polylysine in a system containing partially lysed fibrinogen and thrombin, described in the experimental part, may therefore be due to a clot promoting effect of the basic polyamino acid on remaining small amounts of intact fibrinogen, the concentration of which is too small to be clotted by thrombin within the time of observation. The possibility that polylysine enhances precipitation of partially hydrolyzed fibrinogen molecules in the presence of thrombin cannot be excluded.

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# STUDIES ON THE ACTION OF PAPAIN ON PROTEIN FEEDS*) **)

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## INTRODUCTION

A great deal of progress has been made by A. Fodor and other research groups in the investigation of the behaviour of proteolytic enzymes with peptides and comparatively simple and well defined proteins. Their work served as a basis for our studies on the mode of action of proteolytic enzymes on animal feeds, and the formation of intermediary breakdown products. The present report deals with the action of papain on protein feeds of plant and animal origin.

Since the mode and extent of the action of papain depend on the pH of the environment as well as on the preliminary activation of the enzyme[1,2], the substrates (protein feeds of plant and animal origin) were digested by nonactivated papain and HCN activated papain in phosphate buffers of pH 5.0 and pH 7.5.

The digests were examined for the rate of liberation of the terminal groups COOH, $NH_2$ and α-amino $NH_2$.

Special attention was paid to the formation of a precipitate by the addition of trichloroacetic acid (TCA) to papain digests. The authors reported in two preliminary communications[3,4] a characteristic difference between plant and animal proteins. Pancreatic digests of plant protein feeds, but not of animal protein feeds, yielded considerable precipitates on addition of TCA. In this communication experiments on this differentiating property in papainic digests of the two groups of feeds are described.

## EXPERIMENTAL

### *Materials and methods*

The substrates for the action of papain were fish meal, meat meal, soybean oil cake and peanut oil cake. They were ground to powder, defatted by ether extraction and dried at room temperature.

The papain powder (B.D.H.) was dissolved in M/15 phosphate buffers pH 5.0 and pH 7.5. The activation of the papain by HCN was done according to Bamann et al.[5]

The proteolysis was carried out according to Melnick et al.[6], Pader et al.[7] and Evans[8]. Samples of 4 g of feed were placed into 200 ml Erlenmeyer flasks. 200 mg of papain and 100 ml buffer were added. Blanks containing respectively substrate + buffer (with and without HCN) and papain + buffer (with and without HCN) were prepared in order to examine the degradation of the substrate and the enzyme under the same conditions. After the addition of toluene the Erlenmeyer flasks were incubated at 37° — 38°C for 24 hours.

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### *Examination of the digests*

After incubation, the samples were filtered and the N content of the residue was determined.

Determinations of the following groups were carried out in various parts of the filtrate:

(a) Carboxyl groups (COOH) by the Soerensen[9] formol titration.

(b) Amino groups ($NH_2$) by the Pope-Stevens[10] copper method.

(c) Amino groups by Van-Slykes nitrous acid gasometric method (apparatus with modification by Koch[11]).

NOTE: Full agreement was found between the results obtained by methods (b) and (c).

(d) $\alpha$-amino groups by Van-Slykes[12] ninhydrin method.

To half of the filtrate (50 ml), previously acidified by HCl (methyl red), 12.5 ml TCA 5% were added. The precipitate obtained was washed with warm water containing 0.5% TCA and its nitrogen content was determined by Kjeldahl digestion.

### *Calculation*

The results obtained from the determinations of the terminal groups in the acid hydrolysates of the defatted feeds served as a basis for the calculation of percent hydrolysis. the acid hydrolysis values being taken as 100%. The acid hydrolysates were obtained by refluxing 10 g feed in 100 ml 6 N HCl for 24 hours[13].

The total nitrogen content of the residues and TCA precipitates was calculated on the basis of the nitrogen content of the defatted feeds as 100%.

## RESULTS

The data in Table I show the percentages of terminal groups COOH and $NH_2$ in the acid hydrolysates of the examined feeds and the total nitrogen content of those feeds.

TABLE I

*Percentage of terminal groups COOH and $NH_2$ in the acid hydrolysates and total N content of the various feeds*

| | *Fish meal* | *Meat meal* | *Soybean oil cake* | *Peanut oil cake* |
|---|---|---|---|---|
| Free COOH | 24.4 | 22.5 | 13.7 | 17.1 |
| Free $NH_2$ | 7.9 | 8.3 | 5.2 | 6.2 |
| Total N | 11.2 | 10.7 | 7.0 | 9.5 |

Table II summarizes the results of the proteolytic experiments in which the substrates were digested by the same amount of papain (nonactivated and activated by HCN) in buffer solutions of pH 5.0 and pH 7.5.

The $\alpha$-amino nitrogen liberated by papain from the various digests was not included in the table because the quantities were very small in all cases (about 2% of the $\alpha$-

TABLE II

*Amounts of terminal groups COOH and $NH_2$ in 24 hour papainic digests of different feeds (as % of the total terminal groups in the respective acid hydrolysates)*

| *pH* | *Enzyme* | *Terminal group* | *Fish meal* | | *Meat meal* | | *Soybean oil cake* | | *Peanut oil cake* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Exp.* | *Blank* | *Exp.* | *Blank* | *Exp.* | *Blank* | *Exp.* | *Blank* |
| 5.0 | nonactivated papain | $NH_2$ | 12.3 | — | 11.7 | — | 20.5 | — | 42.0 | — |
| ,, | ,, | COOH | 9.4 | — | 8.6 | — | 19.0 | — | 40.8 | — |
| ,, | HCN activated papain | $NH_2$ | 19.8 | — | 14.1 | 1.2 | 26.3 | — | 45.0 | 3.2 |
| ,, | ,, | COOH | 17.0 | — | 11.1 | — | 22.6 | — | 43.0 | — |
| 7.5 | nonactivated papain | $NH_2$ | 13.0 | — | 5.6 | — | 10.4 | — | 27.9 | — |
| ,, | ,, | COOH | 14.0 | — | 7.1 | — | 14.7 | — | 33.8 | — |
| ,, | HCN activated papain | $NH_2$ | 25.4 | — | 22.9 | 2.9 | 34.0 | 3.3 | 61.8 | 5.6 |
| ,, | ,, | COOH | 20.9 | — | 18.6 | — | 24.9 | — | 53.7 | — |

NOTE: No internal breakdown of the papain was observed.

amino nitrogen liberated on acid hydrolysis) and did not exceed the experimental error[14]. The experimental conditions distinctly affected the amount of free terminal groups liberated in papainic digestion. The effect of the activation of papain by HCN, as measured by the terminal groups liberated, was much more evident in digests at pH 7.5 than at pH 5.0; thus more terminal groups were liberated by the action of activated papain at pH 7.5 than at pH 5.0, whereas the opposite was true for nonactivated papain. A slight deviation was observed when fish meal was digested with nonactivated papain. The action of papain, under the same experimental conditions, varied for the different substrates. The feeds investigated were attacked by the papain in the descending order listed: peanut oil cake, soybean oil cake, fish meal and meat meal. The differences were significant.

The $COOH/NH_2$ ratios for the various papainic digests were not equal to 1. This ratio was not affected by the substrate but was affected by the experimental conditions. Thus in the digests at pH 5.0 with HCN activated and nonactivated papain and in the digests at pH 7.5 with HCN activated papain the ratio $COOH/NH_2 < 1$ whereas in the digests at pH 7.5 with non activated papain the ratio $COOH/NH_2 > 1$.

Table III shows the nitrogen content of the residues and TCA precipitates of various papainic digests.

It was obvious from the blanks that the N content of the residue depended not only on the enzyme activity but also on the substrate solubility in the buffer. The diminution of the N content of the residue in digests obtained with HCN activated papain both at pH 5.0 and pH 7.5 was probably due to the higher solubility of protein caused by HCN, as a similar phenomenon was found in the blanks. The substrate + buffer blanks had generally a higher residual N content than the substrate + buffer + HCN blanks. The different response of plant and animal feeds to pancreatic digestion as shown by the addition of TCA[3,4] was found in the papainic digests also. TCA yielded no precipitates when added to papainic digests of animal feeds or their blanks.

TABLE III

*Total N content of the residues and TCA precipitates (as % of the total N content of the defatted feeds)*

| pH | Enzyme | Total N cont. of the: | Fish meal | | Meat meal | | Soybean oil cake | | Peanut oil cake | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Exp. | Blank | Exp. | Blank | Exp. | Blank | Exp. | Blank |
| 5.0 | nonactivated papain | residue | 71.8 | 97.0 | 48.7 | 67.0 | 51.2 | 95.2 | 24.2 | 79.0 |
| " | " | TCA precip. | — | — | — | — | 8.0 | 4.2 | 5.3 | 4.2 |
| " | HCN activated papain | residue | 32.3 | 87.0 | 33.3 | 56.3 | 35.9 | 75.0 | 22.0 | 59.6 |
| " | " | TCA precip. | — | — | — | — | 6.3 | 2.9 | 3.2 | 2.2 |
| 7.5 | nonactivated papain | residue | 65.0 | 99.0 | 47.2 | 73.0 | 41.4 | 78.3 | 21.3 | 30.9 |
| " | " | TCA precip. | — | — | — | — | 27.7 | 18.5 | 21.8 | 46.0 |
| " | HCN activated papain | residue | 45.8 | 87.0 | 28.9 | 77.4 | 18.5 | 47.0 | 11.6 | 41.0 |
| " | " | TCA precip. | — | — | — | — | 15.7 | 32.8 | 4.2 | 31.6 |

On the other hand, precipitates with considerable N content resulted when papainic digests of plant proteins at pH 7.5 were treated with TCA. A slight precipitate was also obtained on addition of TCA to nonactivated papainic digests of feeds of plant origin at pH 5.0. A much smaller precipitate was obtained from plant protein digests when HCN activated papain was used.

## DISCUSSION

The absence of free $\alpha$-amino acids from the papainic digests is typical of the action of this enzyme. This is in agreement with the results of Winnick[14], who found that papain releases only small quantities of free amino acids from casein, after a long period of digestion. In this respect the action of papain resembled that of pepsin, which released only small quantities of free amino acids from proteins. Pancreatin, on the other hand, released considerable quantities of amino acids.

The methods for increasing the nutritional values of proteins based on the predigestion by papain, like those based on the predigestion by pepsin, may be explained by the transformation of the proteins to peptones. A technical method for raising the nutritional value of proteins by predigestion with papain was developed by Weizmann[17].

The extent of action of papain is greatly affected by the nature of the substrate and in this respect the action of papain differs from that of other proteolytic enzymes. When the four substrates used in this work were digested by papain under the same conditions, different amounts of free terminal groups were released (see Table II), whereas peptic and pancreatic digests of these substrates yielded almost identical percentages of these free terminal groups[15]. This effect is in accord with the fact that the optimal pH for papain activity changed with the substrates[1]. There is a distinct difference in the dependence of HCN activated as against nonactivated papain on the pH of the medium. The main difference between the action of activated and nonactivated

papain lies in the fact that whereas digestion with nonactivated papain liberated more free terminal groups at pH 5.0 than at pH 7.5, the opposite was true for HCN activated papain (see table II).

The results obtained strongly suggest that at pH 5.0 HCN increases the activity of papain and at pH 7.5 it appears to have a different effect on the mode of action. The following considerations support this assumption:

1) The ratio $COOH/NH_2$ in the digests where nonactivated papain was used at pH 7.5 was $< 1$, whereas in the activated digests at pH 7.5 as well as in the other papainic digests examined $COOH/NH_2 > 1$.

2) The average number of amino acids in the peptide molecules of nonactivated papainic digests at pH 7.5 was found to be greater than the average number of amino acids in the peptide molecules of the HCN activated digests at the same pH (Table IV).

The calculation of the average number of amino acids in the peptide molecules of the papainic digests (filtrates) was made according to the following formula:

$$\text{Average number of amino acids in the papainic hydrolysates} = \frac{100 - \text{Total N in the residue}}{\text{Free } NH_2\text{–N in the enzymatic hydrolysate} - \text{Free } \alpha\text{-amino N in the enzymatic hydrolysate}}$$

This formula is an approximation, as it does not take into consideration the dibasic and dicarboxylic amino acids. It merely gives an average value of respective numbers of amino acids in peptides contained in different digests.

Table IV summarizes the average number of amino acids in the peptide molecules of the various papainic digests as calculated with the aid of the above formula.

TABLE IV

*The average number of amino acids in the peptides of the papainic digests*

| *pH* | *Papain* | *Fish meal digest* | *Meat meal digest* | *Soybean oil cake digest* | *Peanut oil cake digest* |
|---|---|---|---|---|---|
| 5.0 | Nonactivated | 2.5 | 4.5 | 2.5 | 2 |
| " | HCN activated | 3.5 | 5.0 | 2.5 | 2 |
| 7.5 | Nonactivated | 3 | 9.5 | 5.5 | 3 |
| " | HCN activated | 2 | 3 | 2.5 | 1.5 |

It appears from Table IV that the activation of papain with HCN at pH 5.0 does not cause any remarkable change in the average number of amino acids in the peptide molecules of the digests. However, at pH 7.5 the average number of amino acids calculated for the peptides of the HCN activated papainic digests is lower than the number calculated when nonactivated papain was used. Hence at pH 5.0 the activation of papain by HCN in all the digests causes the liberation of a greater number of peptide molecules of the same average number of amino acids. On the other hand, at pH 7.5 the HCN activated papain causes a breakdown of the peptides present to smaller ones, but not to free amino acids. These arguments are also supported by comparison between the N content of the TCA precipitates of the various digests (see Table III).

It is known from the work of Butler and coll.[16] and from results found by Bondi and Birk[15] that TCA precipitates peptides containing a large average number of amino acids.

The conclusion that the use of HCN activated papain causes, at pH 7.5, the breakdown of peptides to smaller molecules is supported, too, by the results of nitrogen determinations in the TCA precipitates of those digests. The activation of papain by HCN at pH 5.0 causes only a slight diminution of the N content in the TCA precipitates, which is generally very low in the nonactivated papainic digest at that pH value.

The results obtained concerning the difference between activated and nonactivated papain, especially at pH 7.5, conform with Willstaetter's[18] assumption that activated and nonactivated papain are two different enzymes[19].

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# PECTOLYTIC ENZYMES IN TOMATOES

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In connection with studies on the use of local tomatoes for the production of tomato puree some observations on pectase activity in tomatoes have already been described[1]. We showed then that pectase is present in the clear serum as well as adsorbed on pulp. This is contrary to observations on orange juice where the whole pectase is adsorbed on pulp particles. Indeed a simple qualitative experiment easily shows that orange pulp has better adsorptive capacity for pectase than tomato pulp: if pectase-active clear tomato juice is shaken with, or filtered through, orange pulp, the filtrate is completely inactive.

As pectinase is also known to occur in tomatoes[2], it seemed of interest to see whether it accompanies pectase in all cases. This is important to know also because tomato pectase has been repeatedly recommended as a deesterifying agent for the production of low methoxy pectin[3,4].

Furthermore we wanted to see whether surfactants which are known to inhibit tomato pectase and pectinase[5] would do so with enzyme extracts obtained in various ways.

It was also intended to study the reversible alkaline inactivation of pectinase described for fungal pectinase by Rahman and Joslyn[6], in view of the fact that pectolytic enzymes of the tomato have been repeatedly compared with analogous preparations of fungal origin[2,5].

## MATERIALS AND METHODS

Three enzyme extracts were prepared: *A*, *B* and *C*. *A* and *B* are the clear serum and an alkaline extract of pulp, respectively[1]. *C* is a NaCl extract and was obtained as follows: tomatoes were macerated and the macerate kept until pulp and serum had separated. The pulp was further freed of liquid by filtration through cloth and a saturated NaCl solution was added to the pulp to make up to the original weight of the macerate. The mixture was stirred intermittently for 2 hours and then filtered through cloth. The filtrate was slightly yellowish, but completely clear. It contained 18% NaCl. Care must be taken not to use too high a concentration of NaCl. Extracts made in a similar way but containing 26% NaCl, showed no pectase or pectinase activity.

Pectase activity was measured qualitatively only: 10 cc of a 1% pectin solution, 2 cc of an enzyme extract and a few drops of a 2% $CaCl_2$ solution were mixed in a test-tube and neutralised to Methyl Red. Presence of pectase caused a red colour to appear and jelly to form. All three enzyme extracts were pectase-active. Through addition of a commercial detergent of the alkyl-aryl-sulfonate type (AMA) all three were inhibited.

Pectinase activity was measured by the decrease in viscosity of a sodium pectate solution at pH 5. Measurements were also made at pH 8.2 and 9.1, to examine eventual

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alkaline inhibition. Viscosities were measured as flowing-out times of an Ostwald pipette at 30°C with a water value of 24 seconds. Zero time valuses were obtained by measuring the same mixtures, in which the enzymes had been desactivated previously (20 minutes in a boiling water bath in a closed test-tube).

To remove calcium, a saturated ammonium oxalate solution was added to some of the mixtures to a final concentration of 0.8% ammonium oxalate.

TABLE I
*Pectinase in tomato serum*

*10 cc 2% Na-pectate solution*
*1 cc Enzyme* A
*pH 5 (HCl)*

| *with oxalate* | | *without oxalate* | |
|---|---|---|---|
| *Time (hours)* | *Viscosity (seconds)* | *Time (hours)* | *Viscosity (seconds)* |
| 0 | 62.2 | 0 | 118.5 |
| 6 1/2 | 61.2 | 24 | 111.5 |

TABLE II
*Pectinase in alkaline tomato extract*

*10 cc 2% Na-pectate solution*
*1 cc Enzyme* B
*pH 5 (HCl)*

| *with oxalate* | | *without oxalate* | |
|---|---|---|---|
| *Time (hours)* | *Viscosity (seconds)* | *Time (hours)* | *Viscosity (seconds)* |
| 0 | 61.8 | 0 | 102.4 |
| 2 3/4 | 46.9 | 2 | 53.7 |
| 4 3/4 | 38.0 | 24 | 47.7 |
| 6 3/4 | 33.5 | | |
| 0.25% AMA added | | 0.25% AMA added | |
| 0 | 51.4 | 0 | 86.7 |
| 6 3/4 | 51.4 | 24 | 89.7 |

TABLE III
*Pectinase in NaCl tomato extract*

*10 cc 2% Na-pectate solution with oxalate*
*1 cc Enzyme* C 1:5
*pH* 5 *(HCl)*

| *Time (hours)* | *Viscosity (seconds)* |
|---|---|
| 0 | 60.4 |
| 2 | 42.3 |
| 4 | 35.3 |
| 6 1/4 | 32.1 |
| 23 | 29.8 |
| 0.25% AMA added | |
| 0 | 51.8 |
| 23 | 50.6 |

TABLE IV
*Tomato pectinase at various pH*

*10 cc 2% Na-pectate solution with oxalate*
*1 cc Enzyme* B

| | *Time (hours)* | *Viscosity (seconds)* |
|---|---|---|
| pH 5.0 | 0 | 57.4 |
| | 2 | 31.9 |
| | 6 1/2 | 27.4 |
| pH 8.2 | 0 | 61.6 |
| | 6 | 61.0 |
| pH 9.1 | 0 | 60.3 |
| | 6 | 59.2 |

TABLE V
*Desactivation of tomato pectinase in boiling water*

*10 cc 2% Na-pectate solution with oxalate*
*1 cc Enzyme* C 1:5
*pH 5 (HCl)*

| *Deactivation time* | 10′ | 20′ | 30′ | 40′ | 50′ | 60′ |
|---|---|---|---|---|---|---|
| *Viscosity in seconds after* 0 hrs | 58.2 | 58.8 | 57.6 | 58.8 | 58.8 | 58.5 |
| ” ” ” ” 22 ” | 56.4 | 60.4 | 59.8 | 60.6 | 61.2 | 60.2 |

Enzyme *C* was diluted in a ratio 1:5 in order to prevent flocculation of the Na-pectate.

## RESULTS AND DISCUSSION

The data in Tables I, II and III show that pectase desorbed from pulp through addition of salt or raising of the pH (enzyme *B* and *C*) is accompanied by active pectinase. Only the pectase of the juice (enzyme *A*) is free from pectinase, a fact which should be taken into consideration for the production of high-grade low-methoxy pectin. Tables II and III also show the inhibition of pectinase by a surfactant. According to Table IV, tomato pectinase is inhibited at pH 8 and 9 like fungal pectinase. The heat resistance of tomato pectinase in strong salt solution[7] could not be confirmed, at least under the conditions in Table V.

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# PROTEASES OF THE EGGS OF THE DESERT LOCUST (*SCHISTOCERCA GREGARIA* FORSKÅL)

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It has been found previously[1] that glycerol extracts of diapause eggs of the Moroccan Locust (*Dociostaurus maroccanus* Thnb.) did not hydrolyze casein and gelatine but readily attacked peptones of these proteins as well as leucylglycylglycine, leucylglycine, and chloroacetyltyrosine. Casein and gelatine were also not hydrolyzed by extracts prepared in a mixture of 30 volumes of 88% glycerol, 10 volumes of phosphate buffer (pH 6.8) and 60 volumes of water, whereas such extracts readily cleaved peptones of these proteins. The eggs of *D. maroccanus* are laid in May and develop slowly until September when diapause begins. No conspicuous morphological differentiation takes place during the diapause. From October until May the development of the eggs may be restored when they are kept under moist conditions at an appropriate temperature. In experiments carried out in March, egg pods of *D. maroccanus* were held under moist conditions at 27°—30°. Hatching usually began within 10—12 days. No hydrolysis of casein was obtained with extracts prepared at the beginning of this treatment, whereas a distinct cleavage was observed with extracts made a few days before hatching. Similar results were obtained with diapause eggs of the silkworm (*Bombyx mori* L.). Here, too, extracts of the eggs cleaved peptones and peptides, whereas a cleavage of gelatine and casein was observed only with extracts prepared a few days before hatching[2].

The object of the present paper was to compare the proteolytic activity of diapause eggs as described above with the activity of eggs of a locust which develop without natural diapause. We used eggs of the Desert Locust, *Schistocerca gregaria* Forskål. These eggs were obtained from a local breed of a locust swarm which reached Israel early in 1954. The eggs of *S. gregaria* develop without any interruption or diapause within 12—50 days according to the temperature. In our experiments, freshly laid eggs of *S. gregaria* were kept under moist conditions at 26.5°. They develop and hatch then during 14—21 days. The action of glycerol extracts of such eggs of different ages on casein was investigated. It was found that extracts prepared during the first days after laying attacked this protein to an extremely small extent, if at all. A distinct hydrolysis of casein was obtained with 8 day and older eggs. This proteinase activity increased strongly shortly before hatching.

As to the exopeptidase activities, no cleavage of leucylglycylglycine was obtained with extracts of 1—2 day old eggs of *S. gregaria*, whereas a strong activity towards this peptide was found in extracts made 5 days after laying and in older eggs.

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The different stages of the embryonic development of *D. maroccanus* and *S. gregaria* have been described elsewhere[3,4] Detailed studies on the correlation between these stages and the appearance of various proteases in the developing eggs are under way. However, the results obtained so far seem to indicate that a distinct proteinase action appears in the eggs of *S. gregaria* in an earlier stage of development than in the eggs of *D. maroccanus*.

## EXPERIMENTAL

*Glycerol extracts.* The eggs were washed with water, dried on filter paper and ground with 10 parts of 90% glycerol. The suspensions were kept overnight at 30° in a stoppered flask. After centrifuging and filtration through glass wool, the extracts were kept in the refrigerator.

*The proteinase activity* was tested by Anson's method[5]. To the glycerol extracts an equal volume of 0.1 molar phosphate buffer, pH 8, was added. This diluted enzyme solution was added to an equal volume of a 1% alkaline (pH 8) solution of casein. The reaction mixture was kept at 36° under toluene for 72 hours. After addition of trichloroacetic acid and centrifuging, 1 ml of the filtered supernatant solution was tested colorimetrically (Coleman spectrophotometer, wave length 720 mμ) using the phenol reagent of Folin and Ciocalteu[6]. The values obtained with corresponding reaction mixtures at the beginning of the experiment were subtracted. No increase in colorimeter readings was obtained with controls containing the enzyme without casein or casein alone. The colorimeter readings were compared with those obtained with known solutions of tyrosine. Typical results are given in Table I.

The *exopeptidase activity* was measured by Sorensen's formol titration method with *DL*-leucylglycylglycine as substrate. A 1% solution of the peptide in 0.05 molar phosphate buffer, pH 8, was used. To 10 ml of this solution 2.5 ml of the glycerol extract, diluted 1:1 with 0.1 molar phosphate buffer, were added. Five ml samples were removed at the beginning of the experiment and after 72 hours of incubation at 36° under toluene were titrated with 0.05 N NaOH. The results obtained after subtraction of the blanks are given in Table I.

TABLE I

*The action of glycerol extracts of developing eggs of* Schistocerca gregaria *Forskål on casein and leucylglycylglycine*

| *Age (days)* | *Stage of development** | *Action on casein*** | *Action on leucylglycylglycine**** |
|---|---|---|---|
| 1—2 | I—II | 0.1 or less | none |
| 5 | IX—X | 0.1 or less | 2.00 |
| 8 | X—XII | 0.4 | 3.1 |
| 9 | XI—XX | 0.4 | — |
| 11 | XX | 0.4 | 3.1 |
| 17 | shortly before hatching | 1.8 | 2.8 |

* Corresponding to the stages desribed by Bodenheimer and Shulov[3] for the eggs of *Dociostaurus maroccanus* Thnb.

** Increase in colour intensity with the Folin-Ciocolteu phenol reagent, expressed in micromoles tyrosine.

*** Increase in titration values, ml of 0.05 N NaOH.

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# LEAD TARTRATE, BASIC COMPLEXES, THEIR BEHAVIOUR, COMPOSITION AND STRUCTURE

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Lead tartrate and its behaviour in basic solutions has been studied repeatedly by different workers. However, the constitution of the different compounds obtained requires further investigation[1]. This paper is a continuation of our study on lead citrate[2].

## EXPERIMENTAL

The "Heterometer" (=photometer adapted for the study of suspensions) and the heterometric method have been described elsewhere[3]. The conductivity measurements were made with a "Serfass conductivity bridge Model R.C.M. 15". The conductance cell has been described in a previous paper[4]. A Beckman Model H2 pH meter was used for the pH-titrations. All titrations were made at room temperature (18°—25°C). The conductometric titrations were made at 25° ± 0.1°C. The chemicals used were all C. P. Baker's Analysed.

## RESULTS AND DISCUSSION

### *Heterometric titrations*

Heterometric titrations were carried out in water solution by the addition of sodium tartrate (0.1 M) to solutions of lead nitrate (0.1 M) (=direct titrations), or by the addition of lead nitrate (0.025 M) to solutions of sodium tartrate (0.025 M) (= reverse titrations). In all titrations, precipitation began almost with the first drops of titrant added. In the direct titrations, the points on the abscissa of the maximum optimal density gave formulas which varied with the concentration of the components. In the reverse titrations (Figure 1) a maximum optical density point was obtained at the molar ratio of $2[Pb^{2+}] : 3[Ta^{2-}]$. At this point the optical density values dropped abruptly, following which they remained almost constant. The sudden drop may have been caused by a physical change in the character of the precipitate. A series of heterometric titrations were made either in the presence of NaOH (Figure 2) or by the addition of NaOH to mixtures containing sodium tartrate and lead nitrate. On the addition of lead nitrate (Figure 2), a *clear* solution was at first obtained. At a well defined point on the abscissa the initial precipitation occurred. The density curves rose abruptly and a point of maximum optical density was obtained (curves 4—7). The density values then became constant. Both critical points (point of the *initial* precipitation and of the *first* optical density maximum) were used for further calculations. In Table I are compiled the results of a series of such titrations. In experiments 1—3 the maxima obtained were too high to be measured, and more dilute solutions were therefore used for these determinations (exp. 4—8). Other titrations were made by the addition of sodium tartrate to suspensions

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TABLE I

*General composition*: a *ml* $Na_2$-*tartrate* + b *ml* NaOH + (20-a-b) *ml* $H_2O$ + X *ml* Pb $(NO_3)_2$

| *Experiment number* | *Composition (ml)* $Na_2Ta$ | NaOH | *Used (ml* $Pb(NO_3)_2$*)* *at begin. of precipitation* | *at end of precipitation* | *Calculated (ml* $Pb^{2+}$ *in solution) as:* PbTa′ *only* | PbTa″ *only* | $Pb_2Ta''$ *only* | $Pb_4Ta_3''$ | *ml* $Pb^{2+}$ *required for precipitation of* $Pb_2Ta''$ + PbTa |
|---|---|---|---|---|---|---|---|---|---|
| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) |
| 1 | 8 | 10 | 7 | | 8 | 5 | 10 | 6.7 | |
| 2 | 6 | 10 | 6.8 | | 6 | 5 | 10 | 6.7 | |
| 3 | 10 | 6 | 4.3 | | 6 | 3 | 6 | 4.0 | |
| 4 | 3 | 5 | 3 | 5.8 | 3 | 2.5 | 5 | 3.2 | 5+0.5 = 5.5 |
| 5 | 3 | 5 | 3 | 5.8 | 3 | 2.5 | 5 | 3.2 | 5+0.5 = 5.5 |
| 6 | 5 | 3 | 2 | 6.8 | 3 | 1.5 | 3 | 2.0 | 3+3.5 = 6.5 |
| 7 | 4 | 5 | 3.2 | 7.0 | 4 | 2.5 | 5 | 3.2 | 5+1.5 = 6.5 |
| 8 | 3 | 6 | 3.6 | 6.5 | 3 | 3 | 6 | 4.0 | 6+0 = 6.0 |

Concentrations: exp. 1—3 = 0.1 M; exp. 4—7 = 0.05 M

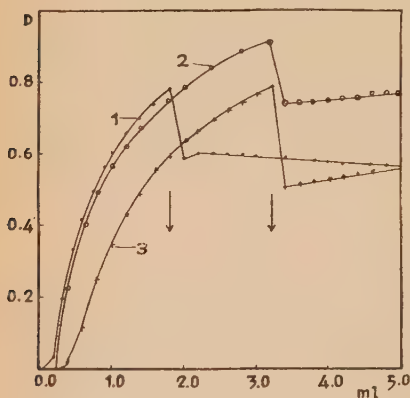



Figure 1

1. 3 cc 0.1 M $Na_2Ta$ + 17 cc $H_2O$ + + X cc 0.1 M $Pb(NO_3)_2$
2. 5 cc 0.1 M $Na_2Ta$ + 15 cc $H_2O$ + + X cc 0.1 M $Pb(NO_3)_2$
3. 5 cc 0.05 M $Na_2Ta$ + 15 cc $H_2O$ + + X cc 0.05 M $Pb(NO_3)_2$

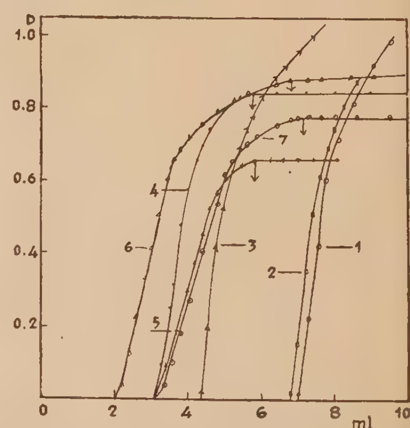



Figure 2

1. 8 cc 0.1 M $Na_2Ta$ + 10 cc 0.1 M NaOH + + 2 cc $H_2O$ + X cc 0.1 M $Pb(NO_3)_2$
2. 6 cc 0.1 M $Na_2Ta$ + 10 cc 0.1 M NaOH + + 4 cc $H_2O$ + X cc 0.1 M $Pb(NO_3)_2$
3. 10 cc 0.1 M $Na_2Ta$ + 6 cc 0.1 M NaOH + + 4 cc $H_2O$ + X cc 0.1 M $Pb(NO_3)_2$
4. 3 cc 0.1 M $Na_2Ta$ + 5 cc 0.1 M NaOH + + 12 cc $H_2O$ + X cc 0.1 M Pb $(NO_3)_2$
5. 3 cc 0.05 M $Na_2Ta$ + 5 cc 0.05 M NaOH + + 12 cc $H_2O$ + X cc 0.05 M $Pb(NO_3)_2$
6. 5 cc. 0.05 M $Na_2Ta$ + 3 cc. 0.05 M NaOH + + 12 cc $H_2O$ + X cc 0.05 M $Pb(NO_3)_2$
7. 4 cc 0.05 M $Na_2Ta$ + 5 cc 0.05 M NaOH + 11 cc $H_2O$ + X cc 0.05 M $Pb(NO_3)_2$

of lead hydroxide (obtained by adding two parts of NaOH to one part of lead). The suspensions dissolved completely and the sodium tartrate required was either *one* tartrate per *one* $Pb^{2+}$ or even less. Lead tartrate suspensions could be dissolved in NaOH but the endpoints were not certain enough.

Analysing Table I we find the following: if columns (3) and (4) are compared, it can be seen that the molar ratio of $[Pb^{2+}] : [OH^-]$ is approximately 2 : 3 at the point of initial precipitation. Assuming that the NaOH is used up for the neutralisation of *one* or *both* hydroxy groups of the tartrate anion, there would be obtained in solution one of the following anion complexes: $[Pb_2Ta'_3]^{5-}$, $[Pb_2Ta''Ta']^{3-}$ or $[Pb_4Ta''_3]^{4-}$. (Ta or $Ta^{2-}$ = tartrate anion; $Ta'$ = $Ta^{2-}$ minus *one* hydrogen from the hydroxy group; $Ta''$ = $Ta^{2-}$ minus *two* hydrogens from both hydroxy groups). The maximum points are well defined and show that the reactions occurring in solution and during the precipitation are of a quantitative character (compare columns (5) and (10)).

### *pH-titrations*

pH-titrations were carried out parallel to the heterometric titrations. The composition was identical to Exps. 1—3 of Table I. The results can be seen in Figure 3. The upper inflexion point at pH ~9.5 lies near the point of initial precipitation. A second inflexion point at pH $\geqq$ 7.0 is obtained at the end of the precipitation. pH titrations in similar concentrations were carried out by the addition of NaOH to suspensions of lead tartrate and an inflexion point at $\leq$ pH 10 was obtained.

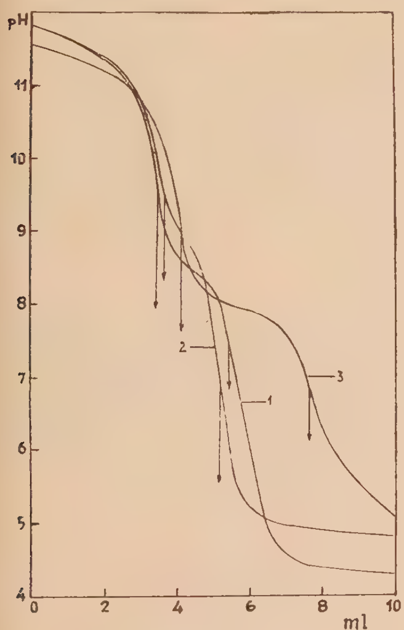



Figure 3

1. 8 cc. 0.1 M $Na_2Ta$ + 10 cc. 0.1 M NaOH + 2 cc. $H_2O$ + + X cc. 0.2 M $Pb(NO_3)_2$
2. 6 cc. 0.1 M $Na_2Ta$ + 10 cc. 0.1 M NaOH + 4 cc. $H_2O$ + + X cc. 0.2 M $Pb(NO_3)_2$
3. 10 cc. 0.1 M $Na_2Ta$ + 6 cc. 0.1 M NaOH + 4 cc. $H_2O$ + + X cc. 0.1 M $Pb(NO_3)_2$

In order to investigate the pH conditions necessary for the precipitation of lead tartrate from alkaline solution and its dissolution, parallel pH and heterometric titrations were carried out by a gradual addition of nitric acid to alkaline solutions of lead tartrate. The different concentrations of the components and the acid were chosen with the purpose of studying the partial reactions which take place. Figure 4 shows the results of two heterometric (curves 1 and 3) and two pH (curves 2 and 4) titrations. The phenomena involved are much too complicated and do not permit a detailed analysis of the

curves. Generally we may represent the partial reactions as follows: on addition of acid, at first the excess of alkali is neutralised and the solution contains $[Pb_4Ta''_3]^{4-}$. Between pH ~9.5 and pH ~8.0, $Pb_2Ta''$ is precipitated. Between pH ~8 and pH ~5, the latter is transformed into the insoluble regular PbTa, which redissolves between pH ~5 and pH ~4.

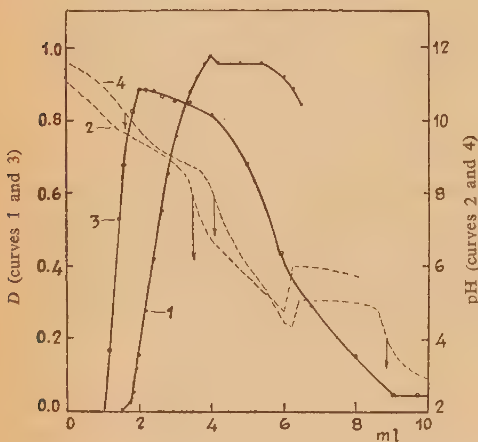



Figure 4

1. 4 cc 0.1 M $Na_2Ta$ + 9 cc 0.1 M NaOH + 4 cc 0.1 M $Pb(NO_3)_2$+3 cc $H_2O$+X cc 0.1M $HNO_3$ (Het.)
2. 4 cc 0.1 M $Na_2Ta$ + 9 cc 0.1M NaOH + 4 cc 0.1 M $Pb(NO_3)_2$+3 cc $H_2O$+X cc 0.1M $HNO_3$ (pH)
3. 4 cc 0.1 M $Na_2Ta$ + 9 cc 0.1M NaOH + 4 cc 0.1 M $Pb(NO_3)_2$+3 cc $H_2O$+X cc 0.2M $HNO_3$ (Het.)
4. 4 cc 0.2 M $Na_2Ta$ + 9 cc 0.2 M NaOH + 4 cc 0.2 M $Pb(NO_3)_2$+3 cc $H_2O$+X cc 0.2 M $HNO_3$ (pH).

As to the existence of a soluble complex of the composition PbTa″, the following may be said: by the titration of an alkaline tartrate solution with lead, there may be obtained a *soluble* intermediate $[PbTa'']^{2-}$ which on further addition of lead is transformed into the soluble $[Pb_4Ta''_3]^{4-}$. However, no breaks which would correspond to the formation of PbTa″ were obtained.

*Conductometric titrations*

The results of the titrations are given in Figure 5. Two breaks are obtained in both curves. If we assume that the soluble lead tartrate complex has the composition $[Pb_4Ta_3'']^{4-}$, then the first break which occurs almost at the beginning of the precipitation would correspond almost quantitatively to the formation of this anion complex. The second break then corresponds to the calculated sum of two insoluble salts $[Pb_4Ta''_3]Pb_2$ + PbTa.

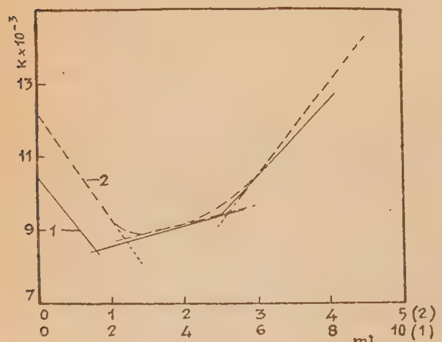
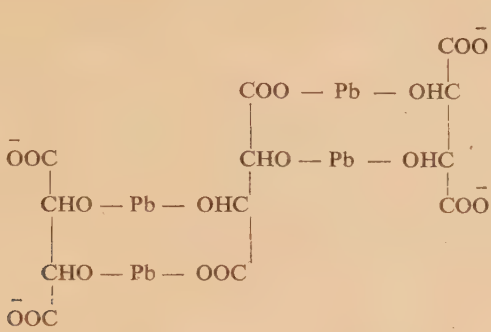



Figure 5

1. 10 cc 0.1 M $Na_2Ta$ + 6 cc 0.1 M NaOH + + 34 cc $H_2O$ + X cc 0.25 M $Pb(NO_3)_2$
2. 8 cc 0.1 M $Na_2Ta$ + 10 cc 0.1 M NaOH + + 32 cc $H_2O$ + X cc 0.5 M $Pb(NO_3)_2$

*Structure*

The structure of $[Pb_4Ta_3'']^{4-}$ may be presented as:

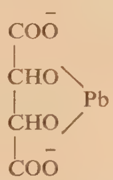


I

Nothing certain can be said concerning the extent to which the carboxylic groups are bound to the lead atoms.

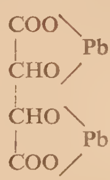
The anion complex obtained has the properties of a dicarboxylic acid and gives an insoluble salt of the composition $Pb_2[Pb_4Ta_3'']$.

Another presentation of the insoluble $Pb_2Ta''$ may be given if the existence of an anion complex $[PbTa'']^{2-}$ is assumed:



II

On addition of lead we may then obtain the insoluble salt $Pb[PbTa'']$ of the dicarboxylic acid, or the anion may be transformed into $[Pb_2Ta'']^0$:



III

However, our experiments give no evidence of the existence of $[PbTa'']^{2-}$. In addition, structure (III) does not emphasize the salt character and the insolubility of $Pb_2Ta''$.

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# MEASUREMENTS OF "EFFECTIVE CRIMP DIAMETER"

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## GENERAL

The presence of crimp in fibres allows for the spinning of more cohesive webs and slivers. Moreover, crimped fibres enable the production of bulkier fabrics, which can trap a considerable amount of air. Accordingly, the quality estimation of wool depends to a high degree on this property, and a great effort has been made by the rayon industry to impart this property to artificial fibres.

In spite of its importance, crimp has not been investigated sufficiently. Some authors (all mentioned in ref. 1 — a recent survey of their work), have tried to define the characteristic wave-lengths of different fibres in relation to their crimp. Others have presented the fibre either as a uniplanar sine-wave or as sine-waves, elliptical curves or screw shaped waves arranged in three dimensions.

It seems, however, that these presentations do not account sufficiently for the statistical side of crimp, which is mainly a random phenomenon, even of "regularly crimped" merino wool, but certainly in the general case.

In the following, some first measurements are reported of what it is proposed herewith to call "effective crimp diameter".

This figure is defined in the following way:

Measurements of shape of (differently loaded) fibres were executed, with the help of a vertically moving comparator, containing a horizontal ocular micrometer. Horizontal coordinates of fibre points were measured at constant vertical intervals of $h$ (ranging from 0.5 to 2 mm). For the calculation of results the curved fibre was treated as a polygonal line.

Using linear regression analysis, the "Effective Crimp Diameter" $D_{eff}$ was calculated as:

$$D_{eff} = 2\sqrt{\sum_{i=1}^{N} y_i^2 / N}$$

where $\sum_{i=1}^{N} y_i^2$ is the minimum sum of squares of the distances of the corners of the polygon from their linear regression line, and $N$ the number of measurements.

The figure $D_{eff}$ corresponds in some way to the "effective voltage" of an irregular alternating voltage, which is generally defined in a similar way.

Degreased "Scotch Blackface" wool fibres were used. All experiments were carried out in an air conditioned room of 65% RH and 20°C.

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## EXPERIMENTAL

$D_{eff}$ of four fibres (A, B, C, D) loaded with 0 and 0.5 g, of two fibres (A and B) with 1 g, and two fibres (C and D) with 4 gm were measured, using 2 mm intervals along 5 cm of fibres.

The mean values of these measurements are given in Table I:

TABLE I

| *Load in gm* | 0 | 0.5 | 1 | 4 |
|---|---|---|---|---|
| I | 4.43 | 0.76 | 0.58 | 0.16 |
| II | 2.90 | 0.49 | 0.16 | 0.02 |
| III | 3.61 | 0.27 | 0.21 | 0.12 |

$D_{eff}$ given in mm.

Between measurements I and II the fibres were held at constant maximal load (1 gm or 4 gm) for 14 days, between II and III at zero load for 14 days.

The figures point, as seen in Figure 1, to a considerable hysteresis of the crimp under these conditions.

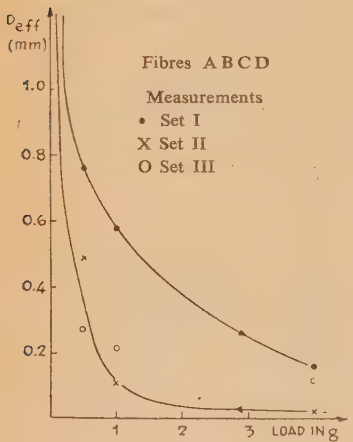


Figure 1

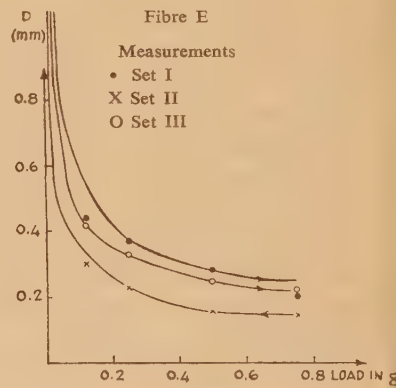


Figure 2

The crimp diameter depends of course strongly on the load applied in stretching the fibre. The "crimp diameter" for zero load on the other side is highly undefined, as real zero load cannot be reached experimentally in any case, and seems to reach asymptotically to relatively high values. Measurements with smaller loads were therefore carried out on fibre E with the results given in Figure 2.

Replication of the above measurements made at 0.5 mm intervals gave, for the same part of fibre and for the same fibre plane, a coefficient of scattering of less than 5% — which is more than satisfactory for this purpose.

All measurements were carried out in one plane only — assuming that no distinguished crimp plane exists in normal fibres. In this case,

$$\sum_{i=1}^{N} r_i^2 = \sum_{i=1}^{N} (x_i^2 + y_i^2) \approx 2 \sum_{i=1}^{N} y_i^2 \tag{1}$$

In another series of measurements (Table II) the variation of "crimp diameter" between different planes of the same fibre segment and under the same load was measured. The coefficient of variation (100 × standard deviation/mean) did not exceed 18% in this series of measurements. The above assumption (1) seems therefore to afford a sufficient basis for the present measurements.

TABLE II

| *Fibre* | *First plane* | *Second plane* | *Third plane* | *Mean* $m$ | *Standard deviation* $\sigma$ | $100\sigma/m$ |
|---|---|---|---|---|---|---|
| G | 0.32 | 0.29 | 0.20 | 0.27 | 0.05 | 18.5 |
| H | 0.41 | 0.30 | 0.44 | 0.36 | 0.06 | 16.6 |
| I | 0.22 | 0.25 | 0.23 | 0.23 | 0.01 | 4.3 |
| J | 0.25 | 0.24 | 0.26 | 0.25 | 0.01 | 4.0 |
| K | 0.27 | 0.26 | 0.25 | 0.26 | 0.01 | 3.8 |
| L | 0.29 | 0.26 | 0.27 | 0.27 | 0.01 | 3.7 |

$D_{eff}$ given in mm.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the advice given by Dr. H. Muhsam on the statistical aspect of this work.

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# SPECIFICITY OF *D*-AMINO ACID OXIDASE

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The action of *D*-amino acid oxidase on *N*-alkyl substituted amino acids was the subject of several investigations. Keilin and Hartree[1], working with extracts of acetone dried kidneys of ox, pig or sheep, found that *DL-N*-methylalanine was oxidized, whereas *DL-N*-methyltyrosine and *DL-N*-dimethylalanine were not. Handler, Bernheim and Klein[2] obtained a considerable oxidation of *DL-N*-monomethylalanine by broken cell suspensions of rat kidneys. They found that monomethyl derivatives of other amino acids were either attacked to a very low extent or not at all. The oxidative action of aqueous extracts of acetone dried pig kidney on several *N*-alkyl derivatives of *DL*-alanine was investigated by Karrer and Appenzeller[3]. These authors found that *DL-N*-methylalanine was oxidized to the same extent as *DL*-alanine, *DL-N*-ethylalanine was oxidized to a much smaller extent, whereas *DL-N*-butylalanine, *DL-N*-dimethylalanine and *DL-N*-acetylalanine were not attacked.

In the present series of experiments we found that aqueous extracts of acetone dried pig kidney did not attack the *N*-ethyl, *N*-hydroxyethyl and *N*-cyclohexyl derivatives of *DL*-leucine, *DL*-valine and *DL*-phenylalanine to any significant extent. On the other hand, it was found that such derivatives markedly inhibited the oxidation of these three amino acids. In some cases the derivatives of one amino acid inhibited the oxidation of the parent compound less than the oxidation of the other amino acids.

*DL*-leucinamide was not attacked by *D*-amino acid oxidase, but it inhibited the oxidation of *DL*-leucine, *DL*-valine and *DL*-phenylalanine by the enzyme.

We found that *L*-amino acids (*L*-leucine, *L*-valine and *L*-phenylalanine), when added in a molar ratio of 5:1, did not inhibit the oxidation of the corresponding *DL*-amino acids. Similar results were described by Keilin and Hartree[1].

The method used by us for the determination of *D*-amino acid oxidase activity, viz. the manometric measurement of the oxygen uptake, actually represents a combined result of two reactions[4]: one is the action of the amino acid oxidase proper, according to the equation:

$$\underset{\displaystyle NH_2}{R.CH}.COOH + O_2 + H_2O \rightarrow R.CO.COOH + NH_3 + H_2O_2 \tag{1}$$

The second reaction is the decomposition of hydrogen peroxide by the catalase, which is present in crude enzyme preparations, the overall reaction being:

$$\underset{\displaystyle NH_2}{R.CH}.COOH + \tfrac{1}{2}O_2 \rightarrow R.CO.COOH + NH_3 \tag{2}$$

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We had, therefore, to reckon with the possibility that our derivatives did not inhibit reaction (1), but rather activated the catalase. Such an activation would lead to an apparent decrease in oxygen uptake, which also might be interpreted as an inhibition of reaction (1). To check this possibility we carried out a series of separate experiments on the catalase activity of our enzyme extracts. It was found that several amino acid derivatives tested (*DL-N*-hydroxyethylvaline, *DL-N*-ethylleucine, *DL-N*-ethylphenylalanine, *DL*-leucinamide) did not influence this activity at all. Our derivatives inhibit therefore the action of *D*-amino acid oxidase proper.

Our experiments show that when one of the hydrogen atoms of the amino group of valine, leucine and phenylalanine is substituted by an ethyl, hydroxyethyl or cyclohexyl radical, the resulting compounds are practically not attacked by the enzyme. The results obtained with leucinamide indicate that a free carboxyl group is required for the oxidation. If one assumes that an inhibiting effect requires the combination of the inhibitor with the enzyme, it follows from our experiments that in the case of *D*-amino acid oxidase the substitution of either amino or carboxyl groups does not preclude such a combination.

## EXPERIMENTAL

N-*alkyl derivatives* of amino acids were prepared by treating $\alpha$-bromo acids with a 33% aqueous solution of the various amines. Ethyl and isopropylamine derivatives were incubated for several days at 37°, hydroxyethyl and cyclohexylamino derivatives were refluxed for 2 hours. The reaction mixtures were then evaporated to dryness in vacuo, and purified by recrystallization from aqueous alcohol or alcohol. The *N*-cyclohexylamino derivatives, which are rather insoluble, were purified by dissolving in acid and re-precipitating by neutralization*.

*Enzyme preparation*: Aqueous extracts of acetone dried pig kidney were used[5]. For each experiment 1 part of the powder was shaken with 40 parts of water for 10 minutes and centrifuged for 15 minutes.

*Buffer*: Pyrophosphate buffer (M/10), pH 8.3, prepared after Warburg and Christian[6] as recommended by Krebs[4] was used.

*Experimental procedure*: The experiments were carried out in a Warburg respirometer at 38° by the usual technique for 60 minutes. The main compartment of the vessel contained 1.0 ml enzyme preparation and 1.2 ml of buffer**, or the mixture of 1.0 ml enzyme, 1.0 ml amino acid derivative (M/10 dissolved in buffer) and 0.2 ml of buffer solution. The side arm contained 0.3 ml of the amino acid (M/15). In the experiments testing the action of the enzyme on the various amino acid derivatives themselves, the latter were added from the side arm.

Oxygen uptake of the enzyme preparation itself was practically zero. The pH of the substrates and analogues was adjusted to the appropriate value. Differences in pH of

* The derivatives of *DL*- phenylalanine were prepared by Mrs. Ruth Zur.

** In experiments with *DL*-valine, half the amount of enzyme extract and 1.7 ml of buffer solution were used, because of the comparatively strong action of the enzyme on this amino acid.

the various reaction mixtures did not exceed 0.2. Such differences in pH do not alter the results, as was found by experiments with the amino acids as sole substrates at pH values varying between 8.0 and 8.6. Typical results are summarized in the Tables.

The catalase activity of our extracts was also measured in the Warburg apparatus. Instead of the *DL*-amino acids, solutions of hydrogen peroxide were used. The concentration of hydrogen peroxide corresponded to the maximum which could be formed by the oxidation of the amino acids tested, or to half of this amount.

TABLE I

*The action of* D-*amino acid oxidase on amino acid derivatives*

| *Substrate* | $O_2$ *uptake* (*in microlitres per* 60 *min.*) |
|---|---|
| *DL*-leucine | 120 |
| *DL-N*-ethylleucine | 7 |
| *DL-N*-hydroxyethylleucine | 8 |
| *DL-N*-cyclohexylleucine | 10 |
| *DL*-valine | 107 |
| *DL-N*-ethylvaline | 6 |
| *DL-N*-hydroxyethylvaline | 4 |
| *DL-N*-cyclohexylvaline | 7 |
| *DL*-phenylalanine | 106 |
| *DL-N*-ethylphenylalanine | 10 |
| *DL-N*-hydroxyethylphenylalanine | 9 |
| *DL-N*-cyclohexylphenylalanine | 6 |
| *DL*-leucinamide hydrochloride | 6 |

TABLE II

*Inhibition of* D-*amino acid oxidase by* N-*substituted amino acids and by leucinamide*

| *Substrate* | $O_2$ *uptake* (*in microlitres per* 60 *min.*) |
|---|---|
| *DL*-leucine | 120 |
| *DL*-leucine + *L*-leucine | 118 |
| *DL*-leucine + *DL-N*-ethylleucine | 24 |
| *DL*-leucine + *DL-N*-cyclohexylleucine | 95 |
| *DL*-leucine + *DL-N*-ethylvaline | 67 |
| *DL*-leucine + *DL-N*-hydroxyethylvaline | 66 |
| *DL*-leucine + *DL-N*-hydroxyethylphenylalanine | 25 |
| *DL*-leucine + *DL-N*-cyclohexylphenylalanine | 22 |
| *DL*-valine | 107 |
| *DL*-valine + *L*-valine | 109 |
| *DL*-valine + *DL-N*-hydroxyethylvaline | 86 |
| *DL*-valine + *DL-N*-cyclohexylvaline | 38 |
| *DL*-valine + *DL-N*-ethylleucine | 88 |
| *DL*-valine + *DL-N*-cyclohexylphenylalanine | 72 |
| *DL*-phenylalanine | 106 |
| *DL*-phenylalanine + *L*-phenylalanine | 105 |
| *DL*-phenylalanine + *DL-N*-ethylphenylalanine | 32 |
| *DL*-phenylalanine + *DL-N*-hydroxyethylphenylalanine | 31 |
| *DL*-phenylalanine + *DL-N*-cyclohexylphenylalanine | 24 |
| *DL*-phenylalanine + *DL-N*-ethylleucine | 85 |
| *DL*-phenylalanine + *DL-N*-hydroxyethylleucine | 85 |
| *DL*-valine + *DL*-leucinamide hydrochloride | 62 |
| *DL*-leucine + *DL*-leucinamide hydrochloride | 77 |
| *DL*-phenylalanine + *DL*-leucinamide hydrochloride | 59 |

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# LETTERS TO THE EDITOR

## Preparation of Benzylcarbamate

Benzylcarbamate was first prepared by heating benzyl alcohol and urea-nitrate at 140°[1], but yields were low. It is usually synthesised by the action of ammonia on benzyl chloroformate[2]. A. M. Paquin[3] prepared it by heating 2 moles of benzyl alcohol with one mole of urea in the presence of $SnCl_4$ for 8 hours, but yields were only 48%. based on the expensive benzyl alcohol used in excess.

In the following, an efficient and cheap method is described, by which this substance can be synthesised in high yields with a maximum reaction time of only one hour.

In a 125-ml round-bottomed flask, equipped with an air-condenser of approximately 1 m length fitted at its top with a glass tube leading into dilute hydrochloric acid for adsorption of ammonia, 54 g (0.5 mole) of *freshly* distilled benzyl alcohol and 30 g (0.5 mole) of urea, dried at 80° are added. The flask is heated on an asbestos wire gauze and the mixture allowed to reflux until white crystals (biuret) are formed. This usually takes from one half to one hour. Heating is now interrupted, as otherwise the benzyl-carbamate formed reacts with excess urea to give benzyl allophanate, thereby lowering the yield of the carbamate. The reaction mixture is poured into 500 ml of cold water under vigorous stirring. Crude benzylcarbamate crystallizes out immediately in white leaflets. After cooling the substance is filtered. Unreacted benzyl alcohol, which separates from the aqueous filtrate, may be recovered, dried over anhydrous sodium sulphate and redistilled. On recrystallization from 20 ml of ethanol, 35—38 g (81—88% based on reacted benzyl alcohol) of a material melting at 85° is obtained. From the mother liquor, an additional portion of benzyl alcohol is recovered by fractionation. Thus a total of 20—24 g of benzyl alcohol is obtained. The benzyl carbamate, which still contains traces of benzyl allophanate, may be further purified by dissolving in ether, separation by filtration of the insoluble allophanate and evaporation of the solvent. Its melting point is thus raised to 86° and the yield is hardly affected by this procedure.

*Anal.* Calcd. for $C_8H_9O_2N$: C, 63.5, H, 5.9; N, 9.3. Found: C, 63.6; H, 5.8; N, 9.3.

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## The Effect of Auxin on the Germination of Lettuce Seeds

The possible significance of auxin in the germination of seeds has long been considered. Kruyt[1] has surveyed the literature on the relation between seeds and auxin. Despite the large amount of work in this field there is still no certainty whether auxin plays a part in germination. Lately[2] it has been suggested that the effect of light during germination is on an auxin-light receptor complex which can be activated or inactivated by red and infrared light respectively. In order to test such an auxin effect and to examine its possible relation to the light-germination interrelationship proposed by Evenari and Stein[3] and Evenari and Neumann[4], the following experiments were undertaken.

It was decided to study whether auxin (indole 3-acetic acid, I.A.A.) would affect the light response of lettuce seeds.

Lettuce seeds variety Grand Rapids* were germinated under varying conditions of light and temperature, with and without I.A.A.

The experiments were carried out as described by Evenari and Neumann[4].

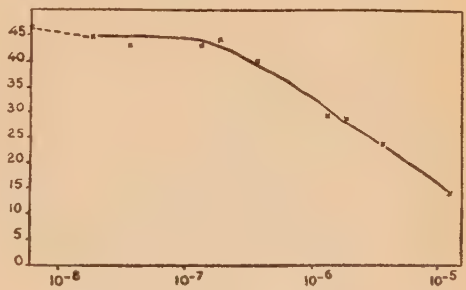


Figure 1

The effect of I.A.A. over a wide range of concentrations is presented in Figure 1. It will be seen that there is no evidence for any stimulatory effect of I.A.A. in germination at 20°C. Identical results were obtained at 26°C, where the dark germination of Grand Rapids seeds is lower than at 20°C.

In continuation, the effect of germination-stimulating red light (R) and inhibitory infrared light (IR) in conjunction with I.A.A. were tested.

The results in Table I are based on four to five replicates, each replicate consisting of 4 Petri dishes containing some 100 seeds. Each result is, therefore, based on some 1500—2000 seeds. Statistical analysis of the differences between the various treatments showed them to be entirely

TABLE I

*Germination of Grand Rapids seeds at 20 °C*

| *Treatment* | *% germination in water* | *S.D.* | *% germination in .01 mg % I.A.A.* | *S.D.* |
|---|---|---|---|---|
| Dark control | 51 | 6.3 | 43 | 5.4 |
| R | 71 | 4.5 | — | — |
| IR | 28 | 5.1 | 34 | 10.3 |
| R+IR | 39 | 6.6 | 57 | 17.5 |

Red light (R), (Corning glass filter 245) — 1/2 mins. at 250 f.c.
Infrared light (IR), (Wratten filter 88 A)—2 1/2 mins. at 500 f.c or 5 mins. at 250 f.c.

non-significant. It can, therefore, be said that I.A.A had no significant effect on germination.

The same results, not presented here, were obtained at 26 °C.

The experiments were repeated at 26 °C, the seeds being placed in the cold, 6—8 °C, for the first three hours of germination (Table II).* No significant effect of I.A.A. could be found.

TABLE II

*Germination of Grand Rapids seeds at 20 °C (after 3 hrs. at 6—8 °C)*

| *Treatment* | *% germination in water* | *S.D.* | *% germination in .01 mg % I.A.A.* | *S.D.* |
|---|---|---|---|---|
| Dark | 49 | 7.5 | 47 | 7.0 |
| R | 68 | 1.3 | 64 | 5.7 |
| IR | 28 | 4.2 | 25 | 3.3 |
| R+IR | 39 | 2.8 | 34 | 5.3 |

Red light (R) — 1/2 min. at 250 f.c.
Infrared (IR) —2 1/2 mins. at 500 f.c. or 5 mins. at 250 f.c.

Experiments with the non photoblastic lettuce seed, variety Progress, at 26 °C, showed that here too there was only inhibition by I.A.A., but at much higher concentrations, 10 mg % being required to cause 50% inhibition.

These experiments give no indication that I.A.A. is involved in the photo-mechanism of photoblastic lettuce seed. Otherwise it would have shown some effect under some of the conditions tested.

But as with all negative experiments it must be admitted, however, that if I.A.A. is present in optimal amounts in the seeds, then any treatment with I.A.A. would only have adverse effects.

Our thanks are due to Miss Gordin and Mr. Ben Shaul for their technical assistance in this work.

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* This pretreatment at low temperatures might augment the auxin effect.

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## Refractometric Control in Ethanol Manufacture by Fermentation

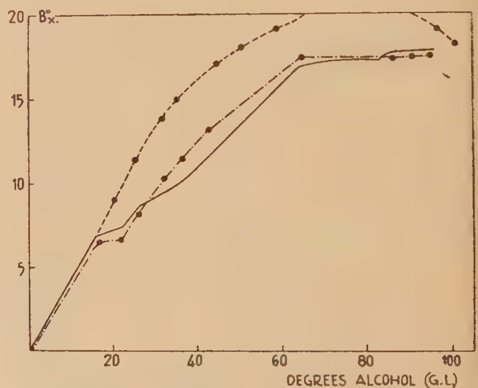
Hydrometric methods are generally used for checking alcoholic fermentation, but their shortcomings are well known. A more accurate and rapid means of control was needed; we therefore tried to work out a method based on the change of the refractive index of saccharine worts in the course of fermentation.

During the conversion of sugar into alcohol, variations in the refractive index are linked to the actual sugar concentration. The relation between sugar concentration (Bx°) and refractive index is known over a wide range of values[1].

The relation between alcohol concentration and refractive index has also been studied, and several values are found in literature[2]. But, for our purposes, it was preferred to prepare a table correlating results obtained with our refractometers to those given by hydrometric readings, as a basis for comparison with results obtained in the same experimental conditions.

It is convenient, in the present case, to express the refractive index in Bx°, according to the instrument's scale.

The refractive index of alcohol-water mixtures of different compositions has been measured by an Abbe refractometer and a Goldberg hand refractometer. Results are reported in Figures 1 and 2.



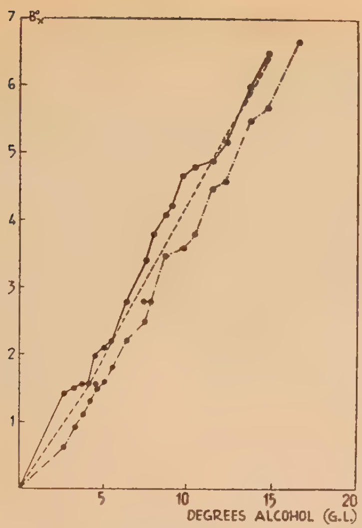

— Abbe refractometer ---- Theoretical curve
—.—.— Goldberg refractometer

Figure 1

At alcohol concentrations normally encountered in ethanol fermentation (up to 9—12% $C_2H_5OH$), Bx° are a direct function of percent alcohol. An increase of nearly 0.35 Bx° is determined by every additional 1% of alcohol. On the basis of these figures, the following equations may be established.

Let us call:

$x_o$ = original Bx° in the wort before fermentation;




— Abbe refractometer ---- Theoretical curve
——— Goldberg refractometer

Figure 2

$x$ = Bx value at each instant during fermentation;

$x_f$ = Bx° after fermentation is completed;

$s$ = non-fermented residual sugar;

$m$ = difference between readings of Bx° and real sugar percent. This difference represents non-sugar soluble solids.

($m$ is characteristic for each wort; for instance: $m = 3.5$—$4°$ Bx in citrus peel pressing-juice, 2—2.5° Bx in sugar-beet diffusion juice; 4° Bx in prickly pear juice. These values are practically constant during the whole work season. It is noteworthy that, in the presence of certain amounts of molasses, $m$ increases enormously).

If fermentation is merely a stoichiometric transformation of sugar in alcohol, and 0.5g $C_2H_5OH$ are obtained for each g of sugar, then:

$$x = {}^1/_2\,(x_0 - m - s)\,0.35 + s + m$$

and $x_f = {}^1/_2\,(x_0 - m)\,0.35 + m$ (assuming that fermentation is completed).

In order to verify the above equations, two sets of experiments were carried out.

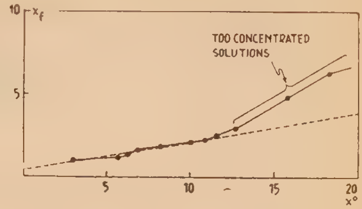
a) *Relation between the initial and the final Bx*

Sugar solutions of different concentration were fermented and the refractive index was measured before and after fermentation.

b) *Bx° at each instant during fermentation*

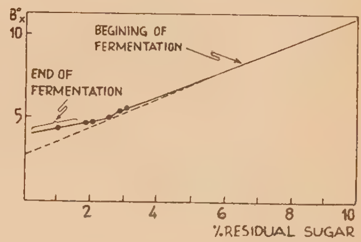
A sugar solution was fermented. The alcohol percent was determined by ebulliometer, the sugar contents by Fehling; samples were drawn at intervals in the course of fermentation and refractometric readings were recorded.

The same was done on saccharine juices of different sources: orange peels, sugar beet, molasses. Results are recorded in Figures 3, 4, 5, 6.




— Experimental --- Theoretical

Figure 3




— Experimental --- Theoretical

Figure 4

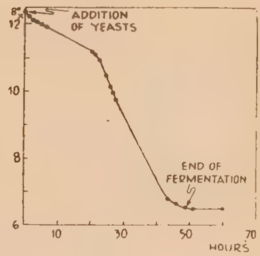
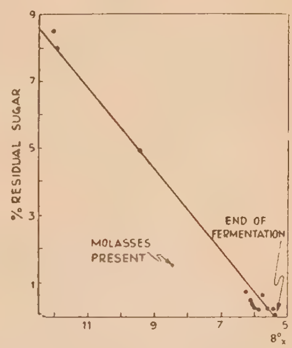



Figure 5




• Experimental — Theoretical

Figure 6

*Practical application of results obtained*

Our equations have been confirmed by results. A practical method for the purposes of alcohol manufacture may now be worked out.

The refractive index variation during fermentation is quick and easy enough to check (see Figure 5).

The completion of fermentation is reached when the refractive index is constant.

For example, let us take the case of orange-peel juice. This juice contains 7.5—9% fermentable sugars and its refractive index corresponds to 11.5—12.5 Bx°. Therefore

$$m \approx 4.$$

When original Bx° = 11.0, final Bx° will be:

$$x'_f = 1/2\,(11 - 4)\ 0.35 + 4 = 5.23.$$

When original Bx° = 13.0, final Bx° will be:

$$x'_f = 1/2\,(13 - 4)\ 0.35 + 4 = 5.58.$$

Since the difference between these two extremes (which, by the way, are very rare) is only 0.35 Bx°, we may assume the average original Bx° to be 12. Final Bx° will be:

$$x_f = 1/2\,(12 - 4)\ 0.35 + 4 = 5.4.$$

The relation between Bx° during fermentation and residual sugar will be given by:

$$x = 1/2\,(12 - 4s)\ 0.35 + 4 + s,$$

or, after simplification:

$$x = 5.4 + 0.82\,s$$

$$s = 1.22\,x - 6.6$$

In ethanol manufacture wort is admitted to fermentation vats when in full fermentation Bx°. should be measured at 1 hour intervals by means of a hand refractometer, accurate enough to allow interpolation of 0.5 Bx°.

If working by "coupage", Bx° should be measured after each addition of fresh juice. When 2 successive readings made at intervals of 1 hour show 5.5 Bx°, a further measurement should be performed in the laboratory by an Abbe refractometer.

If Bx° = 5.5, the juice is to be considered ready for distillation.

If Bx° differs substantially from 5.5, while remaining constant, non fermented sugar should be determined according to Fehling. This may happen in particular cases, or when $m$ is much higher than usual.

Worts in different stages of fermentation have been analysed for sugar, alcohol and Bx°. Results are reported in Figure 6.

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### Carotene in Lettuce Seeds

Lettuce seeds, var. Grand Rapids, have been used frequently in investigations on the light sensitivity of germinating seeds[1]. It has been suggested recently that the mechanism responsible for the light sensitivity is connected with photochemical reactions of carotenes[2]. Since apparently no carotenes have been isolated and identified in lettuce seeds, we have attempted to do so.

Lettuce seeds, var. Grand Rapids (100 g), were first dried and then the whole seeds extracted with petrol ether (b.p. 40—60 °C) in an atmosphere of nitrogen. The extract gave the absorption curve shown in Figure 1, curve A. The extract contained large quantities of fats.

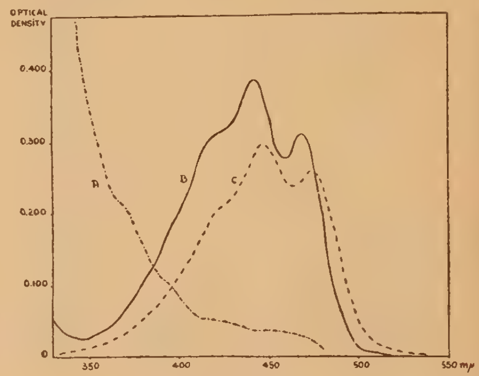


Figure 1

Next the seeds thus treated were ground and once again extracted with petrol ether, in an atmosphere of nitrogen. The extract was concentrated at room temperature *in vacuo* and the residue chromatographed twice on alumina. The almost completely fat free carotene section of the second chromatogram was then cut, eluted with absolute methanol, evaporated *in vacuo* and the residue dissolved in petrol ether. This solution gave the spectrum shown in Figure 1, curve B. The amount of carotene obtained at the end of this procedure from 100 g of seeds was approximately 0.2 mg.

Another lot of seeds (100 g) was dried, ground and saponified for 24 hours at room temperature by shaking in an atmosphere of hydrogen with a solution of 100 g KOH in 250 ml methanol in the presence of 750 ml of petrol ether (b.p. 40—60 °C). The resulting mixture was treated according to the procedure of Karrer and Jucker[3], the ether fraction on evaporation and solution in petrol ether yielding an absorption curve identical in all respects with that shown in Figure 1, curve B. The quantity of carotene obtained by this procedure after saponification was approximately 0.4 mg.

The substance contained in these solutions could not be separated by mixed chromatography on alumina from genuine β-carotene, the absorp-

tion curve of which is shown in Figure 1, curve C. However, as shown in Figure 1, whilst the two spectra are very similar indeed, there is a slight shift in the position of the maxima, indicating that the substance is possibly an isomer of β-carotene[4].

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## The Origin and Maturation of Gametocytes of Plasmodium gallinaceum

Adler and Tchernomoretz[1] concluded that in *P. gallinaceum* gametocytes are derived directly from merozoites emerging from extra-erythrocytic schizonts. The method employed was infecting fowls with sporozoites of *P. gallinaceum* from *Aedes aegypti*, treating the birds daily with doses of 150 mgm per kilo body weight quinine hydrochloride (to repress all erythrocytic schizogony) till blood smears showed infection of erythrocytes with small non-pigmented parasites. It was therefore certain that at this stage all parasites found in the blood smears were derived directly from E. E. schizonts). At this point quininisation was stopped and blood smears were examined at intervals in order to follow the development of gametocytes. It was easy to confirm that gametocytes developed directly from E. E. forms. In some cases morphologically mature sexual forms were found as early as 42 hours after the cessation of quininisation. It was decided to determine at what stage, if any, these gametocytes became functionally mature i. e., capable of infecting mosquitoes *A. aegypti* and producing the whole cycle of development up to and including the presence of sporozoites in the salivary glands. It was found that functional maturity was reached long before the completion of the second generation of erythrocytic schizogony. Thus in one experiment, morphologically mature gametocytes were present 44 hours after the cessation of quininisation, but mosquitoes *A. aegypti* which fed on the bird did not become infected. Four hours later 20 mosquitoes *A. aegypti* feeding on the same bird all became infected, whereas the second generation of erythrocytic schizogony is not completed until 24 hours later. This experiment suggests that functional maturation follows morphological maturation after a short interval — in this case up to four hours. In another positive experiment mosquitoes became infected after they fed on a bird 47½ hours after cessation of quininisation. In some other experiments there was an unexplained delay in the development of both gametocytes and schizonts in the red cells. In one case no mature gametocytes were found 53 hours after cessation of quinine, and mosquitoe feeding experiments were negative.

The above experiments with mosquitoes definitely confirm that *P. gallinaceum* gametocytes are derived directly from E. E. forms as in the case of the genus *Haemoproteus* and allied genera.

I thank Professor S. Adler for suggesting the above problem for my M.Sc. thesis and for his kind advice.

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# NEWS AND VIEWS

## The 15th Annual Meeting of the Israel Chemical Association

The 15th Annual Meeting of the Israel Chemical Association took place on April 20-22nd at the Technion, Israel Institute of Technology, Haifa. In the opening session, greetings from the Prime Minister and many governmental and scientific institutions and organisations were read: The Presidential address, delivered by the outgoing President, Ernst D. Bergmann, was devoted to "Paul Ehrlich's theory and its influence on the development of therapeutic chemistry"; it was preceded by a survey of the state of chemistry in Israel and a number of current problems of interest to scientists in general and to chemists in particular.

A symposium on "Phosphates" was attended by over 250 people, many of whom participated in the lively discussion which followed the lectures given. Dr. Bentor (The Hebrew University of Jerusalem) spoke on "The geology and genesis of the phosphates of the Negev", and A. Katchalsky (The Weizmann Institute of Science, Rehovot) on "The chemistry and physical chemistry of polyphosphates", F. Bergmann (The Hebrew University of Jerusalem) discussed "The structure of the active group in serum cholinestrase", and A. Bondi (Agricultural Research Station, Rehovot) "The importance of phosphorus in animal nutrition".

The original papers read at the meeting were divided into three sections, each of which held three sessions. A total of 55 papers were read. The Israel Chemical Association was fortunate in having as guest lecturers Prof. C. Coryell (Massachusetts Institute of Technology), Prof. I. Fankuchen (Brooklyn Polytechnic Institute) and Prof. D. Rittenberg (Columbia University). All original papers were available to the members of the Association as preprints by the courtesy of the *Bulletin of the Research Council of Israel*; they are being published in the *Bulletin*.

In the business meeting of the Chemical Association, the outgoing President reported on the state of the Association and its current problems. Dr. I. Dostrovsky was unanimously elected President for the next two years, as were members of the Board of Directors: Dr. D. Ginsburg, Dr. D. Lavie, Dr. A. R. Steinherz and Mr. I. Gillon.

## Professor Fodor Honoured by The Hebrew University of Jerusalem on Seventieth Birthday

Professor Andor Fodor, Professor of Biological and Colloidal Chemistry at the Hebrew University was the guest of honour at a large reception held by the University on March 3 to celebrate his seventieth birthday and to mark the occasion of his retirement after 30 years' service.

The President of the University, Professor B. Mazar, who presided at the function, recalled that Professor Fodor was the University's first teacher and that he had been invited by Dr. Chaim Weizmann to organize its Chemistry Institute, which became the cornerstone on which the future Faculty of Science was based. President Mazar paid tribute to Professor Fodor as a great teacher who had trained a whole generation of chemists and research workers who were now in service of the University itself and other scientific institutes, and who had made a highly significant contribution towards the advancement of science in Erez Israel.

Professor M. Bobtelsky, Professor of Inorganic Chemistry, spoke of Professor Fodor as a distinguished scientist with a wide philosophic outlook who had always maintained very high standards despite many difficulties.

The Dean of the Hebrew University-Hadassah Medical School, Professor E. Wertheimer, conveyed the congratulations of the School's Biochemistry Department, which is staffed mostly by young scientists trained by Professor Fodor. The speaker recalled that, though Professor Fodor was still a young man when he left the University of Halle to come to the Hebrew University, he was already recognized in Europe as a scientist of note.

Dr. N. Lichtenstein, Professor in Biochemistry, paid tribute to Professor Fodor not only as a notable teacher, but as an original writer on science against a background of general culture.

In replying to the tributes, Professor Fodor reviewed his long experience in Israel. There had indeed been many difficulties, especially in the early days, but he had always been stimulated and encouraged by the creative spirit which prevailed throughout the University. In particular, he was deeply appreciative of the fact that scientists at the Hebrew University have always been accorded a full measure of freedom of research.

**Scientific Colloquium of the Israel Union for Theoretical and Applied Mechanics**

The fourth scientific colloquium of the Union took place at the Technion-Israel Institute of Technology, Haifa on the 14th of April, 1954.

The meeting was opened by Professor M. Reiner, Chairman of the Association.

Guest of the meeting was Dr. G. W. Scott-Blair of Reading University, England.

The following papers were read:

Prof. S. Goldstein — The mathematics of exchange processes in fixed columns

Dr. G. W. Scott Blair — Psycho-physics of measurement

Prof. M. Reiner — Finite torsion of metal wires.

Mr. Z. Chashin — The elastic constants of solid-solid dispersions

Prof. F. Ollendorff — Wave mechanics of the double layer

Dr. W. Low — Dielectricity in microwaves

Prof. S. Irmay — Analogies between solids and liquids.

The colloquium was well attended and the interest was evident in the discussions.

The Union will be represented by Prof. Reiner at the forthcoming meeting of the General Assembly of the International Union which is to be held in Brussels on the 27th and 28th of July. Prof. Goldstein who is a personal member of the Assembly and of its Council will also be present.

# 15th ANNUAL MEETING OF THE ISRAEL CHEMICAL ASSOCIATION,

April 20—22, Haifa

## I. Physical, Inorganic and Analytical Chemistry

### The Mechanism of Hydrolysis of Esters of Inorganic Oxyacids, Using $O^{18}$ as Tracer, and its Relation to other Reactions in Alkaline Media

The mode of bond fission in the alkaline hydrolysis of a number of esters of inorganic oxyacids has been determined using $O^{18}$ labelled compounds. These included esters of hypochlorous, hypobromous, chloric, bromic, iodic and perchloric acids, as well as nitrites, nitrates, chromates, sulphates and acetates.

The modes of bond fission possible may be written as:

(I) $Y^- + C\text{–}O\text{–}X \rightarrow Y\text{–}C + O\text{–}X^-$ $S_{NC}$

(II) $Y^- + C\text{–}O\text{–}X \longrightarrow Y\text{–}X + C\text{–}O^-$ $S_{NX}$

(III) $Y^- + H\text{–}C\text{–}O\text{–}X \rightarrow Y\text{–}H + C\text{–}O + X^-$ $O_N{=}E$

Each of the above schemes may theoretically proceed either by a bimolecular or unimolecular mechanism. Not all possible mechanisms, however, have been observed. The results are summarised in Table I.

TABLE I

| Ester | Type of Fission | Ester | Type of Fission |
|---|---|---|---|
| tert.-Butyl hypochlorite | II | tert.-Butyl nitrite | II |
| | | Trityl nitrite | II |
| tert.-Butyl hypobromite | II | $n$-Butyl nitrate | (mixed) |
| Trityl hypochlorite | II | tert.-Butyl nitrate | I |
| Trityl chlorate | II | Trityl nitrate | I |
| Trityl bromate | II | di-tert.-Butyl chromate | (mixed) |
| Trityl iodate | II | di-Trityl chromate | (mixed) |
| Trityl perchlorate | I | tert.-Butyl acetate | (mixed) |
| $n$-Butyl nitrite | II | Trityl acetate | (mixed) |

The alkyl groups may be arranged in an order representing increasing tendency to reaction by $S_{NC}$ mechanism.

Phenyl <$n$-alkyl <sec.-alkyl <tert.-alkyl <trityl <hydrogen.

In going along this series, the sequence of changes represented below will occur:

$$S_{NC}1 \rightarrow S_{NC}2 \rightarrow S_{NX}2 \rightarrow S_{NX}1.$$

The point of change from mechanism $S_{NC}$ to $S_{NX}$ (*i.e.* (I)→(II)) will depend upon the nature of the acid. The stronger the acid, the further to the left in the above series will the change in mode of bond fission occur. It is possible in this way to fit the acids examined into the series in the following way:

| Phenyl — | $n$-alkyl — | tert. alkyl — | trityl — | H |
|---|---|---|---|---|
| $HClO_4$ $H_2SO_4$ $HNO_3$ | $H_2CrO_4$ | $HAc$ | $HNO_2$ $HClO$ $HClO_3$ $HBrO_3$ $HIO_3$ | |

The close relation between the mode of bond fission and various reactions of these esters in organic chemistry are fully discussed. Particular attention is devoted to oxidation reactions (III above) which are shown to fall naturally into the scheme outlined. For a tertiary ester to be an oxidizing reagent it is necessary that it hydrolyze by mechanism (II) $S_{NX}$ and that the group $X^-$ be stable.

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### The Formation of Esters of Inorganic Oxyacids

The mechanism of formation of esters of inorganic oxyacids from alcohols can be described by the general relation

$$ROH + XOY \leftrightharpoons ROX + YOH \qquad (1)$$

where R is the organic group and X and Y are the cationic groups of inorganic acids or hydrogen.

With the reactions of acids (Y = H) and alcohols in polar media, two mechanisms can be postulated

$$R\,\vdots OH + H \vdots OX \leftrightharpoons ROX + H_2O \qquad (2)$$

$$RO\,\vdots H + HO \vdots X \leftrightharpoons ROX + H_2O \qquad (3)$$

where either or both of the reacting species may be protonated. In these schemes the breaking of any one of the bonds can be rate determining, giving the usual unimolecular or bimolecular kinetics.

In dilute aqueous solution, weak acids such as $HOCl$, $HNO_2$, $H_2CrO_4$, $HAc$ react by mechanism

(3) as is shown by using alcohols with isotopically labelled oxygen. The reaction has already been shown to be bimolecular for carboxylic acids.

Strong acids in dilute aqueous solution are fully ionised and do not form esters. Under conditions which promote the formation of carbonium ions, esters of strong acids may be formed by mechanism (2). Thus aqueous solutions of nitric acid form esters by both mechanisms. This is verified by kinetic measurements.

Acid anhydrides form esters with alcohols in non-aqueous media. The nature of the ester formed depends on the electron distribution between the two groups X and Y (scheme 1). Thus in the series of the oxides of nitrogen, the esters formed are as indicated:

$N_2O_3$ reacting as $\overset{\delta+}{NO}\ \overset{\delta-}{NO_2}$ forming nitrite esters only

$N_2O_4$ reacting as $\overset{\delta+}{NO}\ \overset{\delta-}{NO_3}$ forming nitrite esters only

$N_2O_5$ reacting as $\overset{\delta+}{NO_2}\ \overset{\delta-}{NO_3}$ forming nitrate esters only.

Using isotopically labelled alcohols, it is found that the bond between the oxygen and carbon atom remains intact, indicating that esters are formed by the cationic part of the molecule analogously to scheme (3).

A similar scheme is proposed for the formation of esters from the oxides of chlorine and sulphur.

With mixed anhydrides the nature of the product is also determined by the electron attracting power of the groups X and Y. In the series of anhydrides containing oxygen, nitrogen and chlorine,

$NOCl$ acting as $\overset{\delta+}{NO}\ \overset{\delta-}{Cl}$ forms nitrite esters only

$NO_2Cl$ acting as $\overset{\delta-}{NO_2}\ \overset{\delta+}{Cl}$ forms hypochlorite esters only

whereas $NO_2ClO_4$ acting as $\overset{\delta+}{NO_2}\ \overset{\delta-}{ClO_4}$ will form nitrate esters only.

On comparing the pairs containing chlorine and nitronium groups, it is evident that the electron attracting power increases in the order

$$ClO_4 > NO_2 > Cl > NO$$

This is in the same order of decreasing acid strength and is also related to the mechanism of the hydrolysis of the esters of the corresponding acids.

Similar schemes are suggested for mixed anhydrides of sulphur, chromium and the reactions of the mixed anhydrides of acetic acid and inorganic oxyacids.

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## The Catalysed Exchange between Methylamines and Hydrogen

It has been shown that the exchange of hydrogen between ammonia and gaseous hydrogen on metals involves the dissociative adsorption of ammonia on the surface[1]. From the rate of formation of the molecules $NH_2D$, $NHD_2$ and $ND_3$ during the exchange reaction between ammonia and deuterium, Kemball[2] suggested that this exchange involves only one hydrogen atom at a time and thus involves an adsorbed amide radical rather than an imide or nitride.

The possibility that a nitride is an intermediate product in the exchange on iron powder is indicated however by the fact that a nitrided iron powder, which does not catalyze the exchange reaction, is rapidly cleared of the surface nitride by conversion to ammonia under the conditions of the exchange reaction.

Further evidence as to the nature of the intermediate was sought by comparing the rate of exchange of deuterium with ammonia, methylamine, dimethylamine and trimethylamine, respectively, on iron powder.

The rate of exchange of $ND_3$ and $(CH_3)_2ND$ with light hydrogen was also measured. The exchange, as measured by the change of the D content of the hydrogen gas, is found to involve primarily the hydrogen atoms directly bound to the nitrogen (three in $NH_3$, two in $CH_3NH_2$, one in $(CH_3)_2$ NH and none in $(CH_3)_3N$).

The rate of this exchange was the same within 10 percent for the different amines and ammonia, as was also the temperature dependence of the exchange reaction in the range of 250 °—330 °C. This provides further evidence for the predominantly stepwise exchange of the hydrogen atoms and for the unimportance of the nitride as an intermediate. The exchange of the hydrogen atoms of the methyl groups was found to be about sixty times slower at 330 °C than that of the N bound hydrogens.

At this temperature some decomposition of the amines might be expected. This very slow exchange was measured accurately by the interaction of $(CH_3)_2ND$ or $(CH_3)_3N$ with deuterium where the more rapid exchange of N-bound hydrogens does not occur.

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### Anion-Exchange Studies of the Formation of Metal Complexes with Anions

The interpretation of the adsorption of metals from complex-formation media with anion A by anion-exchange resin RA is not in as satisfactory a state as that for cation exchangers. It has been shown (Fronaeus 1953) that the adsorption follows the law:

$$(MA_{\nu})_{res.} = K\,(MA_{\nu})_{soln.} \qquad (1)$$

for the metal ion $M^{\nu+}$, and the addition of more A to form higher complexes ($MA_{\nu+1}$, etc.) lowers the gross distribution constant D.

Detailed consideration of the chemical reactions shows that for trace metal concentrations, the data may be explained by the main reaction:

$$(MA_{\nu})_{soln.} + RA = RMA_{\nu+1} \qquad (2)$$

with enhanced binding due to the second reaction:

$$RA + RMA_{\nu+1} = R_2MA_{\nu+2} \qquad (3)$$

which gives no effect on solution components. The authors will show how this interpretation correlates with known stepwise complex-formation constants for various metals $M^{\nu+}$, how it may relate to solvent-extraction studies on the M-A system, and how the intervention of non-complexing anion B helps clarify reaction (3), which should be a very prominent reaction as judged by cation-exchange data.

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### A Study of the Accuracy of Kelvin's Equation for Concave Surfaces in Microscopic Capillaries

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### The Use of Recoil Atoms in the Elucidation of the Structure of Ionised Surfaces

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### The Tensile Strength of Gels

A method and an apparatus have been devised for measuring the tensile strength of fuel gels.

This apparatus can be used for measurements of the tensile strength of almost any visco-elastic gel, of almost all consistencies and fluidities. The measurement of the tensile strength of such materials is useful and is indicated in most cases to supplant measurements of consistency, penetrability, etc, since tensile strength is a physically well defined constant of a very important property and quite independent of the apparatus employed. The determination necessitates no apparatus more complicated or a technique more refined than the conventional consistometers, penetrometers, etc.

For approximative evaluation of the tensile strength, in less general cases, a very simple apparatus for field use can be constructed.

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### The Free Energy of Poly-Ampholytes

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### The Interaction between Poly-Electrolyte Solutions and Divalent Ions

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### The Mechanism of the Polymerisation of Nitro-olefins

The polymerisation of vinyl compounds is a chain process, the chain carrier being a macroradical or a macro-ion. Accordingly, two mechanisms are possible: a free radical, and an ionic one. Whereas the free radical mechanism of vinyl polymerisations has been extensively investigated, there is yet little known about the ionic mechanism of polymerisation. The researches of Polanyi, Plesh, Skinner, et al. suggest, that in these reactions the active chain carrier is a carbonium ion, which is formed as a result of a proton-monomer interaction. The free proton is apparently formed by the dissociation of the catalyst—co-catalyst complex: $AlCl_3 + H_2O \rightarrow HAlCl_3OH \rightarrow H^+ + (AlCl_3OH)^-$.

The termination of the active chains may take place by addition of a negatively charged ion to the growing chain, by loss of a proton and double bond formation (Dainton and Tomlinson)[1] or by cyclization (Bergmann)[2].

Reactions of this sort are characteristic of vinyl monomers having electron releasing groups in the α-position, e.g. two methyl groups (isobutylene). These groups strongly polarize the double bond:

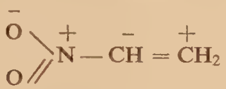
$$\begin{array}{l} CH_3 \searrow \\ \qquad\quad \overset{+}{C} = \overset{-}{CH_2} \\ CH_3 \nearrow \end{array}$$

A proton or a strong electrophilic reagent (Lewis acid) may add to the β-carbon, and thus set free

an effective positive charge on the $\alpha$-carbon for propagation.

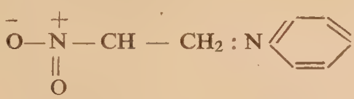
In the present work the question was raised as to whether in the case that electronegative groups were attached to the $\alpha$-carbon, an anionic polymerisation reaction would be possible, and whether general Lewis bases would act as catalysts for its initiation.

In order to investigate this possibility, nitro-ethylene was chosen as monomer;



In this case, bases may join the $\beta$-carbon and set free an effective negative charge, which will act as chain carrier.

The work presented here has shown that the basicity of amines (pyridine and its derivatives) is sufficient in order to form an apparently stable complex with this molecule:



It was shown that under the catalytic influence of these bases, nitro-ethylene undergoes a rapid polymerization at 20 °C in a homogeneous phase (butanone was used as solvent). The rate of polymerization is of first order with regard to catalyst conc. $(C)$ and of second order with respect to monomer conc. $(M)$.

$$\text{Rate} = K(C)(M)^2 \qquad (1)$$

It was also shown — by the use of pyridine derivatives having a substituent in the $\alpha$-position — that the above mentioned complex formation comprises the initiation step. In all the cases where steric hindrance is *avoided* — the Broensted catalysis law is maintained.

$$-\log K = K'pK_B \qquad (2)$$

where $K$ is the overall rate constant.

Equation (2) proves beyond doubt the *ionic character* of this reaction. An additional proof is provided by the fact that this monomer polymerizes at low temperatures.

The experimental results may be accounted for by assuming the following kinetic scheme:

$$M + C \longrightarrow CM^- \quad \text{Initiation}$$

$$CM^- + M \longrightarrow CM^-_2$$

$$CM_n + M \longrightarrow CM^-_{n+1} \quad \text{Propagation}$$

$$CM^-_{n+1} \rightarrow CM_{n+1} \text{ or } CM^-_{n+1} \rightarrow M_{n+1} + C$$

Termination.

It is assumed in this scheme that termination is spontaneous. The exact nature of this step is not yet clear.

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2. Bergmann, E., Taubadel, H. and Weiss, H., 1931, *Ber.*, 64, 1493.

### The Function of Anti-Corrosive Pigments

The pigments in anti-corrosive paints can be divided into three groups:

(a) Pigments that are themselves insoluble in water and that do not give active compounds with the constituents of paints.

(b) Alkaline pigments such as red lead and lead sulphate, that react with constituents of the paints vehicle to give metallic soaps which hydrolyse and protect the metal.

(c) Soluble pigments such as zinc chromate, which are partially soluble in water to give ions which inhibit the corrosion of various metals.

The solubilities of a number of pigments of the last two groups have been examined, relative to the effect of their solutions on the rusting of iron. It has thus been found possible to distinguish those pigments suitable for the production of anti-corrosive paints by a simple test, without the necessity for testing the paints themselves.

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### The Utilisation of Local Phosphates by Alkaline Methods

1. Survey on the methods for the treatment of natural phosphates.

a) Treatment with sulphuric acid to superphosphate.

b) Treatment with alkali by melting or leaching: the most useful proposal seems to be the addition of sodium carbonate and silica.

2. Experiments (at the Institute for Technological Research) in a rotating furnace.

3. Description of the experiments and of the influence of the operating conditions.

4. Composition of the mixture: $3P_2O_5{:}3Na_2O{:}16CaO : 5SiO_2$. The chemistry of the process is described by the following equation:

$$2\,(9CaO.3P_2O_5.CaF_2) + 6Na_2CO_3 + 5SiO_2 =$$
$$= 6\,(Na_2O.2CaO.P_2O_5) + 4\,(2CaO.SiO_2) +$$
$$+ SiF_4 + 6CO_2$$

*Results.*

a) The optimum temperature for the above mixture is 1100—1200 °C.
b) Raw materials containing 20 % $P_2O_5$ give a product with 16—18 % citrate-soluble $P_2O_5$.
c) For 20 % $P_2O_5$, one has to add about 18 % sodium carbonate.

d) The optimum fineness of the raw material is 25 mesh/$cm^2$.

e) The weight of the final product is about equal to that of the raw phosphate.

5. Brief survey on preliminary experiments with the phosphate so obtained in comparison with superphosphate (carried out by Prof. Plaut, Agricultural Research Station, Rehovot, 1942). The quality of the two products is equal: the new product does not affect the plants or leaves adversely.

6. In England it has been found that one can use mixtures of lower sodium content and obtain, in addition to sodium calcium phosphates, calcium silicophosphate. One uses two thirds of the quantity of sodium of the above procedure and works at a higher temperature.

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**The Movement of Potassium and its Adsorption in Ca-soil in Relation to Various Potassium Salts**

The object of this work was to determine the effects of: (a) the type of the anion, (b) its valence, and (c) the pH of the soil, on the diffusion of potassium and its distribution in the soil as soluble, exchangeable and fixed potassium, by setting up experiments allowing diffusion of potassium salts into the soil. The soil chosen for this purpose was a light sandy loam, commonly found along the coastal strip of Israel, and mostly planted with citrus trees. The sample was obtained in the area of the Agricultural Research Station, Rehovot, and, since a homogeneous profile was found in this area, only a single average was taken: 0—50 cm depth.

Before setting up the diffusion experiments, the soil was freed from exchangeable bases such as Mg, K, and Na, by saturating it with a solution of 1N $CaCl_2$. The residual $CaCl_2$ was then removed by washing with dist. water until free from Cl. The soil was then dried, lightly broken up, and passed through a mm sieve. Mechanical, physical and chemical analyses (including pH value, exchangeable bases, water and acid soluble potassium) were made of the original soil. The Ca-soil was introduced into the diffusion chamber containing the soil, in the centre of which there was a compartment containing purified sand. In preliminary experiments, we found that the most suitable quantity of potassium salt solution to be added to the sand compartment, was 5 ml of a normal solution (5 meq. K). Optimum amount of water added to the soil was found to be intermediate between its water holding capacity and its field capacity (23%). The salts used were KCl, $KNO_3$, $K_2SO_4$, a mixture of $K_2HPO_4$ and $KH_2PO_4$, $KH_2PO_4$ alone, $K_2HPO_4$ alone, and $K_2CO_3$. The experiments were arranged by letting each salt stand for one, three and five weeks in order that approximate equilibrium conditions might be reached.

The optimum times of standing and concentration were found by a series of preliminary experiments, i.e. by determining the stages at which approximate equilibrium was reached between water soluble, exchangeable and fixed potassium. It was found that by using 5 ml of a normal solution in conjunction with 46 ml water in 200 g of soil, optimum conditions were secured. In the light of the results obtained, it was shown that approximate equilibrium was generally reached in one to three weeks between water soluble and exchangeable potassium, whereas the max. fixed potassium was obtained only after some five weeks.

From Table II it can be seen that the rate of diffusion of potassium varied a little when neutral salts were used (max. 60.4—72.4% of the K added to the central compartment). Comparatively high results were observed only with $K_2CO_3$, reaching a max. of 85% and not passing farther than the two cells neighbouring the central one, in contrast to the other cases in which potassium ultimately diffused to the farthest cell.

The amount of fixed potassium also varied a little when neutral salts were used, such as KCl, $KNO_3$, $K_2SO_4$, or a mixture of $K_2HPO_4$ and $KH_2PO_4$, averaging 11.25%, irrespective of the change of the type of the anion, namely: $Cl^-$, $NO_3^-$, $SO_4^{=}$, $PO_4^{\equiv}$, or the change of its valence, whether mono-,di-, or trivalent.

It was only when the soil was alkaline, or when alkaline salts were added, that the amount of fixed K became correspondingly larger and larger. Thus in the case of $KH_2PO_4$, where the pH was 6.8, fixed K amounted to 7%, whereas with $K_2HPO_4$ (pH=7.8) it became 13%. The effect of soil alkalinity was even more distinct in the case of $K_2CO_3$ (pH=9.2) where fixed K reached up to 24%.

It would seem, therefore, that raising the soil pH tends to increase the ratio of the fixed to the unfixed K, and to shorten the extent of the K diffusion into the soil. The addition of pH-lowering preparations to K-fertilizers is expected to increase the effectiveness of these fertilizers by cutting down the K fixation and furthering its range of diffusion.

In conclusion, it may be remarked that the above results are evidently related to a given soil type under the given conditions of this work. They do not exclude the possibility of obtaining other results when other types of soils are treated under the same or different conditions of experimentation.

TABLE I

Soil Analysis

| *Mechanical Analysis* | | *Physical Properties* | | | | *Chemical Analysis* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *Exchangeable bases* | | | | |
| *Determination* | % | *Properties* | % | $CaCO_3$ | *pH* | *Bases* | *mg equiv. per 100 g soil* | *Expressed as % of total bases* | *K in aqueous extract 1:5 mg equiv./100g* | *K in HCl extract of soil* |
| Moisture | 2.10 | Water holding capacity | 32.50 | | | Ca | 7.00 | 73.90 | 0.07 | 3.53 |
| Clay | 14.50 | | | | | Mg | 1.74 | 18.40 | | |
| Silt | 2.60 | | | | | K | 0.41 | 4.30 | | |
| Coarse sand | 65.20 | Water field capacity | 13.50 | Trace | 7.7 | Na | 0.32 | 3.40 | | |
| Fine sand | 17.70 | | | | | | | | | |
| | | | | | | | 9.47 | 100.00 | | |

TABLE II

Water soluble, exchangeable and fixed potassium in a Ca-soil brought in contact with various K-salts of different pH values

| *Potassium salts* | pH | *Weeks of diffusion* | *% of the total diffused K*: *Water soluble K* | *Exchangeable K* | *Fixed K* | *Total *K %* |
|---|---|---|---|---|---|---|
| KCl | 7.1—7.3 | 1 | 53 | 40 | 7 | 59.3 |
| | | 3 | 38 | 54 | 8 | 67.4 |
| | | 5 | 37 | 50 | 13 | 70.6 |
| $KNO_3$ | 71.—7.3 | 1 | 53 | 45 | 2 | 58.2 |
| | | 3 | 47 | 50 | 3 | 63.8 |
| | | 5 | 43 | 45 | 12 | 64.4 |
| $K_2SO_4$ | 7.1—7.3 | 1 | 52 | 43 | 5 | 73.2 |
| | | 3 | 46 | 47 | 7 | 70.8 |
| | | 5 | 47 | 41 | 10 | 76.4 |
| $KH_2PO_4$ + $K_2HPO_4$ | 7.1—7.3 | 1 | 37 | 59 | 4 | 66.5 |
| | | 3 | 35 | 56 | 9 | 68.0 |
| | | 5 | 37 | 53 | 10 | 64.4 |
| $KH_2PO_4$ | 6.7—6.9 | 1 | 37 | 60 | 3 | 64.9 |
| | | 3 | 35 | 59 | 6 | 65.0 |
| | | 5 | 37 | 56 | 7 | 60.4 |
| $K_2HPO_4$ | 7.7—7.9 | 1 | 36 | 56 | 8 | 63.4 |
| | | 3 | 34 | 57 | 9 | 72.0 |
| | | 5 | 34 | 53 | 13 | 72.4 |
| $K_2CO_3$ | 9.1—9.3 | 1 | 26 | 51 | 23 | 80.0 |
| | | 3 | 20 | 57 | 23 | 80.0 |
| | | 5 | 20 | 56 | 24 | 85.0 |

* Total *K* diffused into the soil cells out of 5 mg equiv. placed in the central sand compartment.

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## Microdistillation and Boiling Point Determination with Milligram Quantities

In the course of other investigations, it became necessary to fractionate very small quantities of liquids. An apparatus constructed for the purpose can also be used for the quick and accurate determination of boiling points with quantities of 3 mg and less.

The apparatus consists of a pyrex tube (3 mm diameter) whose inner surface has been ground to opaqueness. This tube is connected with a narrower tube (1 mm diameter, 8 cm length) whose far end is sealed off. The sample is introduced into the narrow end of the tube in a capillary, and the remainder of the narrow tube is filled with ground glass. The whole of the narrow tube and about 1 cm of the wider one are inserted into a heating metal block with two thermometers placed very near the two ends of the narrow tube.

The block is heated with a gas burner so that both thermometers show the same temperature. The temperature is raised at a rate of 4—6° per minute until, when it approaches the boiling point, the ground inner surface begins to show a moist spot at a distance of 2—3 mm from the heating block. At the boiling point, the tube becomes quickly transparent first between the spot and the heating block, then in the opposite direction.

It is easy, even without much experience, to determine the boiling point with an error of 1—2°. A large number of determinations have been carried out. Of course, the apparatus can be used at any desired pressure.

In the same apparatus, fractionations can be carried out with small quantities of material; the various fractions can be transported by heating to the more remote parts of the ground tube. Wet filter paper wound around the tube serves as "condenser". If the fractionation serves only analytical purposes, the length of the moist spots formed by the condensation of the vapours can be used for a quantitative determination of the various fractions after suitable calibration. This microdistillation method is subject to only the same limitations as any distillation procedure (azeotropic mixtures, etc.).

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**A simple Counter-current Distribution Apparatus**

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**Determination of Radon and Radium in Water**

An investigation is being made into the nature and detection of radioactive elements in water sources of the country. During this work a method has been developed for measuring the radon and radium content of these waters. This technique has been developed to a stage where it is possible to obtain quick and accurate results during routine analysis.

Samples of water from springs, wells, etc. are collected and stored for a period of two or three weeks during which repeated measurements are made on their radium and radon content. Since radon is a rare gas, it may be quantitatively removed from the water sample.

The following procedure is used: one litre of the water, acidified with HCl, is transferred by suction into an evacuated vertical column packed with glass helices. The dissolved gases which are evolved from the water are dried and transferred to a vessel containing calcium turnings. The calcium is heated to boiling by an induction furnace, when all the gases except the rare gases argon and radon are removed by the calcium. The rare gases containing the radon are then introduced into calibrated Geiger-Mueller counters and the radioactivity is determined.

The radon content of the water at the time of its removal from the source is then calculated from the known half life of radon and its daughters.

The radium is measured in an analogous manner. The water sample, after removal of the radon, is allowed to stand for a period of 20—30 days. The equilibrium concentration of radon is then determined as above.

This procedure has been calibrated using standard radium solutions. The limit of sensitivity of the method is of the order of $10^{-12}$ Curie/litre or $10^{-12}$ g Ra/litre and its reproducibility is better than 10%.

An analysis of the results for radon and radium content from water samples in different parts of the country gave large variations in the ratio Rn/Ra. In the equilibrium state this ratio is 1, whereas values ranging from $\infty$, 200, 100, and 50 were found. The causes for these deviations are not yet clear, and further work is proceeding to clarify this point. A parallel investigation is also being made on the products of disintegration of the uranium series.

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**Microdetermination of Chlorate Ion and Nitroglycerine by Means of Brucine**

1) By means of brucine a simple method has been worked out for the determination of the chlorate ion in ammonium perchlorate up to a concentration of 0.02% chlorate. The sensitivity of the method is as high as 10 μg per ml. Ammonium perchlorate has no effect whatsoever on the determination. The absorption curve of the coloured compound was determined using a DU Quartz Beckman spectrophotometer.

2) Small quantities of nitroglycerine isolated from chromatograms of extracts of propellants are easily determined quantitatively by measuring the absorption of the colour developed with brucine. Sensitivity of the reaction was 25 μg nitroglycerine per ml.

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**The Spectrophotometric Determination of Small Quantities of Water with the Aid of Cobalt Salts**

In the course of a study of the autoxidation of bitumen, it became necessary to determine small quantities of water in bitumen. Gravimetric methods did not come into consideration because of the fluffiness of bituminous materials, and the Fischer method cannot be used because of the reaction of bitumen with iodine.

A spectrophotometric method was therefore developed, based on cobalt chloride. It was known that cobalt salts change their colour from blue to rose, when water is added to their solutions in anhydrous solvents. We used anhydrous alcohol, which does not dissolve bitumen and only to a very slight degree bituminous products which are formed by oxidation, and determined the transmission at 700 mμ in a Beckmann spectrophotometer for varying quantities of water (up to 150 mg/10 ml of alcohol) in presence of cobaltous chloride. With a concentration of 1% of cobalt salt, one can determine 10 mg quantities of water with satisfactory accuracy.

If one works with an alcoholic extract of bitumen, one has to determine the transmission of this extract (without addition of cobalt) and take it as 100%, so as to avoid the possible error caused by the colour which the bitumen imparts to the alcohol.

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### The Determination of Added Inorganic Phosphates in Flour and Bread

The development of *Bacillus mesentericus* in bread, especially during the summer, can be prevented[1] by the addition of calcium hydrogen phosphate. Quantities of 0.4—0.7% (on the basis of the flour) prevent the "ropiness" of bread, and the addition of the phosphate to bread dough is compulsory in this country. A method has been worked out for the determination of phosphate in bread and flour, adapting the procedure of Bell and Doisy[2] and of Briggs[3]: hydroquinone and ammonium molybdate develop a blue colour in presence of phosphate.

It has been shown that this method is sufficiently sensitive and accurate, and that it is possible to elute all the phosphates added to bread and flour.

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## II. *Organic Chemistry*

### The Near Ultraviolet Spectra of Crystalline Naphthalene, Phenanthrene and Durene

The spectra of the above molecular crystals were investigated in order to develop a technique for the direct determination of the symmetry of the wave functions describing the excited states involved in the electronic transitions from the ground state. In the experiments, a Wollaston prism was placed behind the slit of the spectrograph in order to photograph both polarization components simultaneously.

The spectra show a relatively sharp structure at 20 °K. According to present theory each crystal transition in the investigated substances should consist of two components of different polarization.

It was found that in the spectra of naphthalene and phenanthrene only the 0—0 band is split into two components while the electronic-vibrational combination bands seem to originate from a frequency intermediate between the frequencies of the 0—0 band components. The components of the 0—0 band are 100% polarized whereas the remaining bands are only partly polarized. The relative intensity of the polarization components allows determination of the symmetry of the excited electronic states.

In the case of durene the 0—0 band components are not split, and it is possible to explain this fact on the basis of the crystal structure and a simple dipole-dipole interaction model.

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### Separation of Fluoranthene and Chrysene by Cyclic Molecular Distillation

The molecular distillation of a solution of fluoranthene and chrysene in a mixture of poly-ethyleneglycols showed that the elimination temperatures of the two hydrocarbons are sufficiently different to make their separation possible.

From a mixture of fluoranthene and chrysene in the ratio 79:21, the first distillations gave 56% of the fluoranthene with an average purity of 93%;

re-distillation of the chrysene-rich fractions yielded 25% of this hydrocarbon with an average purity of 82%.

This method of cyclic molecular distillation permits a fast separation of polycyclic compounds; it is simpler than repeated crystallisation or the classical distillation methods.

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### The Isotopic Analysis of Oxygen in Organic Compounds

The requirements of the isotopic analysis of oxygen are discussed, and the existing methods are reviewed.

A new method, depending on the equilibration of the sample with carbon dioxide is described in detail.

It was found that compounds which undergo reversible or irreversible dehydration may be analysed for the isotopic composition of oxygen by heating the compound in the presence of carbon dioxide and a catalyst:

$$R-O^* \leftrightharpoons R' + H_2O^* \qquad (1)$$

$$H_2O^* + CO_2 \leftrightharpoons H_2O + COO^* \qquad (2)$$

Alcohols and certain aldehydes, ketones, acids, ethers, esters give this reaction, but differ greatly in the mechanism and the rate of reaction.

Some compounds show an irreversible dehydration, in which case care should be taken in bringing the reaction to completion. Others, such as alcohols, undergo a reversible dehydration—hydration reaction. Alcohols show different behaviour depending on the position of the hydroxyl group. In the case of primary alcohols, reaction (1) occurs between alcohol and its ether whereas tertiary alcohols are in equilibrium with their olefin, and all the oxygen contained in the alcohol exchanges with the carbon dioxide to be analysed.

The experimental procedure consists of heating the sample of one millimole of material with 0.06 millimoles of carbon dioxide in the presence of 1 mg of sulphuric acid in an ampoule of a volume of 1.3 ml. The temperature and time of heating depend on the type of material to be tested. Results of the exchange experiments show that alcohols can be divided roughly into three groups:

A. Primary alcohols require a temperature of 200 °C for one hour;

B. Secondary alcohols require a temperature of 170 °C for one hour;

C. Tertiary alcohols require a temperature of 155 °C for half an hour.

Materials which require temperatures of 180 °C and above for their dehydration reaction must be heated a second time at 160 °C in order to equilibrate the water formed with the carbon dioxide, since at a temperature of 180 °C the amount of liquid water and the solubility of carbon dioxide in the liquid are too small to attain complete equilibrium in a short time. After the heat treatment the equilibrated carbon dioxide is separated from the rest by fractional distillation and introduced into the mass spectrometer for measurement.

It was shown that the equilibration reaction does not proceed by an organic carbonate formation, since no oxygen isotope-exchange was found between ethyl carbonate and carbon dioxide. The experimental facts which prove the dehydration mechanism are:

1. Only compounds giving dehydration reaction, exchange oxygen with carbon dioxide.
2. In a number of compounds the exchange reaction is catalysed by traces of water.
3. In alcohols formation of ether or olefin was found.
4. The exchange reaction is inhibited at extreme conditions of temperature and solubility, where the carbon dioxide—water reaction becomes rate determining.

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### Study of the Mechanism of Hydrolysis of Diethyl Phosphorochloridate Using $O^{18}$

Rate measurements showed that the reactions of dialkyl phosphorochloridates with nucleophilic reagents are of the bimolecular type. Two alternative mechanisms may fit such kinetics, a one-step displacement:

$$H_2O + (EtO)_2P(O)Cl \rightarrow (EtO)_2POOH + HCl$$

or a rapid reversible formation of an intermediate complex, which slowly decomposes to the products'

$$H_2O + (EtO)_2\,P(O)\,Cl \underset{\text{fast}}{\overset{\text{fast}}{\rightleftharpoons}} (EtO)_2\,P\,(OH)_2\,Cl$$

$$\xrightarrow{\text{slow}} (EtO)_2\,POOH + HCl$$

By labelling one of the components with $O^{18}$ it was possible to prove that the reaction proceeds essentially by the first mechanism. On hydrolysing diethyl phosphorochloridate labelled in the phosphoryl group with $O^{18}$, in excess of ordinary water, it was found that the excess water after completion of hydrolysis did not become enriched in $O^{18}$. This proves that the reaction does not proceed through an intermediate complex. The determination of $O^{18}$ in water was carried out by equilibration with ordinary carbon dioxide, and mass spectrometric measurement of the latter.

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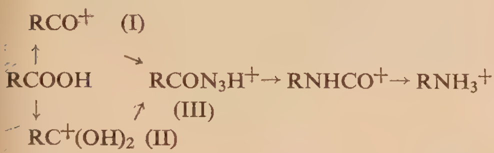
## On the Mechanism of the Schmidt Reaction with Benzoic Acid

In connection with work on transformation reactions of high polymers, we are investigating the mechanism of the Schmidt reaction[1], using some simple carboxylic acids. It has been claimed by various authors[2,3] that the transformation of carboxylic acids into primary amines by means of hydrazoic acid in sulfuric acid solution proceeds via the intermediates indicated in the following scheme:



Acids in which the formation of the carbonium ion (I) is favoured (e.g. 2,4,6-trimethylbenzoic acid), react rapidly, even in the cold; on the other hand, acids which ionize to give mainly the dihydroxy carbonium ion (II) (e.g. benzoic acid), undergo the Schmidt reaction only at temperatures of 35—50 °C. At these temperatures difficulties arise in the kinetic study due to the instability of the hydrazoic acid, particularly when the progress of the reaction is judged by gas evolution. A more complete picture of the course of the reaction is obtained when the concentrations of the postulated intermediate (III), as well as those of the starting materials and the final products, are quantitatively determined at a given moment.

It was found that benzoic acid, benzazide and aniline can be determined spectroscopically by the following procedure: an aliquot sample of the reaction mixture is added to a large excess of water, thus quenching the reaction. A portion of this solution is made alkaline. Since benzazide was found to be fairly stable in dilute acid solution, its concentration can be determined along with that of benzoic acid in the acid solution at 2300 A and 2550 A (Figure 1). In alkaline solution, benzazide decomposes rapidly, and aniline can then be determined at 2800 A and 2900 A (Figure 1).

Using the above analytical method, preliminary results on the Schmidt reaction with benzoic acid in media of varying acidity (water-sulphur trioxide mixtures) at 0 °C were obtained. It was found that in 95% sulphuric acid no reaction occurred. In 100% sulphuric acid, contrary to the findings of Newman and Gildenhorn[2], reaction proceeded at a fair rate. It may be seen from Figure 2 that the concentration of benzoic acid decreased steadily, whilst that of aniline increased correspondingly. The concentration of benzazide was low and practically constant throughout the experiment. The sum of the individually determined concentrations was constant at all times and equal to the initial concent-

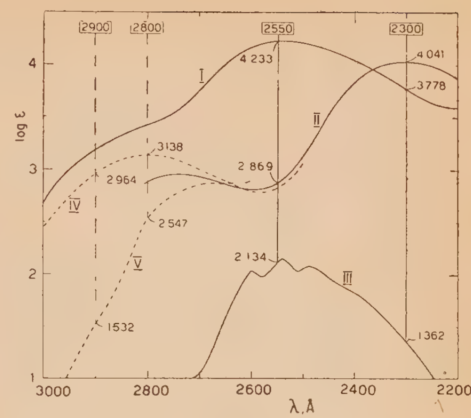



Figure 1
Molar extinction coefficients in the U.V. range of: benzazide in 0.4N $H_2SO_4$ (I); benzoic acid in 0.4N $H_2SO_4$ (II); anilinium ion in 0.4N $H_2SO_4$ (III); aniline in 0.01N NaOH (IV); benzoate ion in 0.01N NaOH (V).

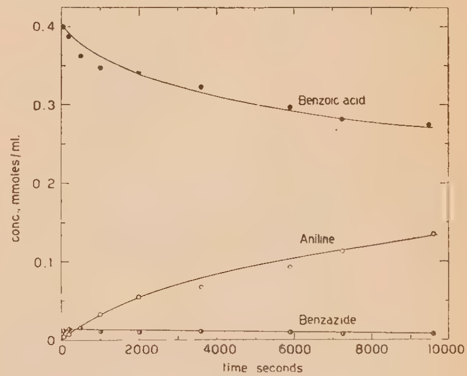



Figure 2
Schmidt reaction with benzoic acid in 100% $H_2SO_4$; initial concentration of hydrazoic acid: 0.5 mmol/ml; $t = 0$ °C.

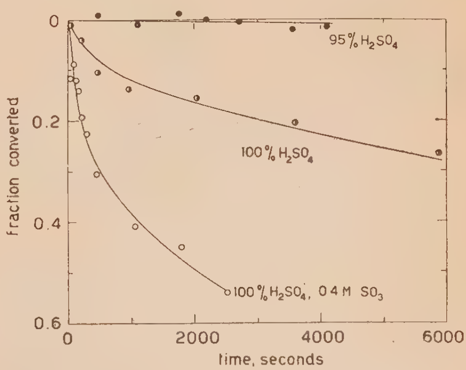



Figure 3
Conversion of benzoic acid in Schmidt reactions at 0 °C. Initial concentration of benzoic acid: 0.4 mmol/ml.

ration of benzoic acid. When the reaction was performed in 100% sulphuric acid to which one mole of sulphur trioxide per mole of benzoic acid had been added, the rate of reaction was much higher. In this case the concentration of benzazide reached a maximum of 12% of the initial benzoic acid concentration at fer 500 seconds and then decreased slowly. The rate of disappearance of benzoic acid from the reaction mixture in the three above experiments, is given in Figure 3.

In order to evaluate the kinetic constants of the various steps of the Schmidt reaction, further knowledge of the decomposition of benzazide in sulphuric acid solutions is required. In a preliminary experiment, it was found that in 100% sulphuric acid solution (at the freezing point of the solution), benzazide disappeared almost immediately: 0.3 mmol in 20 ml sulphuric acid yielded 75% benzoic acid and 25% aniline. This result indicates that the formation of benzazide under the conditions of the Schmidt reaction is reversible and that excess of hydrazoic acid may be required to complete the transformation into aniline.

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## Studies on the Michael Reaction

The unusual activity of the α-hydrogen atom in α-amino acids, which expresses itself in their relatively easy racemisability and in their aldol-like condensation with aromatic aldehydes, led to an attempt to use α-amino-acid esters in the Michael reaction. The condensation of an amino acid (arginine) with fumaric acid has been observed as an enzymatic process, but involves the amino-group and not an α-hydrogen atom[1]. Also *in vitro*, such a reaction has recently been observed[2,3]: esters of α-amino-acids are cyanoethylated at the nitrogen atom by acrylonitrile.

The methyl esters of *N*-acetyl or *N*-benzoyl-α-phenylglycine react with acrylonitrile (or methyl acrylate) in the following manner:

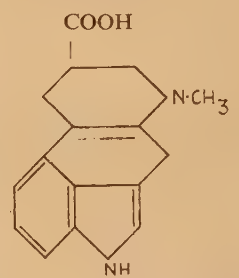
$$CH_2 = CH.CN + C_6H_5.\underset{\displaystyle NH.Ac}{CH}.COOCH_3 \rightarrow C_6H_5.\underset{\displaystyle CH_2{-}CH_2.CN}{C}(NHAc).COOCH_3$$

Under the catalytic influence of Triton B in *t*-butyl alcohol as solvent, acrylonitrile yields methyl α-phenyl-α-acetamino (benzamino) γ-cyano-butyrate.

If one assumes that this reaction has general applicability, certain obscure points in the current theory of the biogenesis of alkaloids become clearer.

(a) In the synthesis of quinine and cinchonamine from the 3-(β-hydroxyethyl)-indole and 3,4-dihydroxyphenylalanine, one has to assume that the α-hydrogen atom of that amino-acid adds to the indole system.

(b) The biosynthesis of lysergic acid (I) from tryptophan (or *N*-methyltryptophan) can best be formulated as a Michael reaction.

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## The Reaction of Ethyl Orthoformate with Aromatic Amines

Ethyl orthoformate, when heated with aniline, is known[1] to give diphenylformamidine and *N*-phenyl formimino ethyl ether. The application of this reaction to other aromatic amines has been studied.

The yields of the substituted formimino ethers increase in the order: *p*-chloroaniline < aniline < *o*-anisidine < β-naphthylamine < α-naphthylamine < *m*-chloroaniline. This order corresponds, except for the case of *p*-chloroaniline, to the order of decreasing basicity, the $pK_b$ values being 9.93[2]; 9.42[3]; 9.51[3]; 9.89[3]; 10.08[3]; 10.4[4] respectively. A direct nucleophilic displacement reaction seems therefore to be excluded and a mechanism initiated by an acidic attack appears to be involved, e.g. via the formation of hydrogen bonds — the existence of hydrogen bonds between amines and ethers has been observed frequently[5], and also the rise of temperature from 21 °C to 27 °C when 6.4 g of *m*-chloroaniline was mixed with 7.4 g of ethyl orthoformate may be ascribed to this effect, as 85% of the starting material can be recovered from this mixture by distillation *in vacuo* at room temperature.

The following reaction mechanism is proposed:

$$RNH_2 + CH(OC_2H_5)_3 \rightleftharpoons (H_5C_2O)_2CH\text{-}OC_2H_5 \xrightarrow[\quad C_2H_5OH]{} \quad \underset{\displaystyle \underset{\displaystyle RNH}{H}}{\downarrow}$$

$$\underset{\text{I}}{RNHCH(OC_2H_5)_2} \xrightarrow[-C_2H_5OH]{} \underset{\text{II}}{RN:CH.OC_2H_5}$$

The intermediate product (I) can not be isolated, but the assumed formula not only explains the formation of (II), but also — by reaction with a second amine molecule — the formation of the diaryl formamidines RN:CH.NH.R (III).

Claisen[1] assumed that in the reaction between aniline and ethyl orthoformate (II) is formed via (III). This can not however, be the only way to (II), since the reaction of ethyl orthoformate with di-*m*-chlorophenyl-formamidine is much slower than that with *m*-chloroaniline and requires a much higher temperature. In accordance with the assumed mechanism, the yield of (II) is increased when the molecular ratio ethyl orthoformate: amine is increased, since the side reactions giving the formamidines are suppressed.

With increasing basicity of the amine, the yield of the diaryl formamidine increases. The explanation suggested is that the formamidines may be formed not only directly from the amines or by an electrophilic attack on the formimino ethers (as shown by the acid catalysis of the reaction between *N*-aryl formimino ethers and amines), but probably also by a nucleophilic attack of the amine on the formimino ether. This latter assumption is supported by the exothermic reaction between the *N*-aryl formimimo ethers and Grignard compounds, which gives aldehydes.

The results of this investigation are given in the following table

| *Amine* | *Moles* $CH(OC_2H_5)_3$: *moles* $RNH_2$ | *Time of reaction* (min.) | *Yield of* $RN:CH.OC_2H_5$ (%) | *Temperature of reaction* (°C) |
|---|---|---|---|---|
| Aniline | 1.1 : 1 | 65 | 30 | |
| Aniline | 3 : 1 | 55 | 73 | |
| *o*-Anisidine | 1.1 : 1 | 60 | 31 | |
| β-Naphthyl-amine | 1.1 : 1 | 60 | 33 | |
| α-Naphthyl-amine | 1.1 : 1 | 55 | 41 | 110 |
| *m*-Chloro-aniline | 1.1 : 1 | 55 | 47 | |
| *m*-Chloro-aniline | 3 : 1 | 45 | 84 | |
| *p*-Chloro-aniline | 3 : 1 | 50 | 48 | |
| *m*-$Cl.C_6H_4N$ : $CH.NH.C_6H_4Cl$-*m* | 1 : 1 | 120 | 10 | 150 |

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## Cholegenin and Iso-cholegenin

In the neutral fraction of ox gall, there were found in addition to a large quantity of cholesterol: a) some pregnane-3α, 20β-diol (identified with the substance of this formula, prepared from progesterone), b) two new compounds, cholegenin and *iso*-cholegenin, which have the formula $C_{27}H_{44}O_4$ and give mono- and diacetates. Cholegenin is transformed into *iso*-cholegenin by heating or treatment with mineral acids.

Cholegenin is 3α, 26-dihydroxy-spirostan, and *iso*-cholegenin differs from it in its configuration at $C_{25}$. This conclusion is based on the following facts:

1) By degradation of cholegenin and *iso*-cholegenin by means of acetic anhydride, oxidation of the product and subsequent saponification, one obtains 20-oxopregn-16-en-3α-ol which was identified by comparison with an authentic specimen.

2) Oxidation of cholegenin diacetate with chromic acid yields 3α-acetoxy-16β-hydroxy-*bisnor*-cholanic acid lactone. The same lactone was obtained by oxidation of 3α-acetoxy-*iso*-spirostane (*epi*-smilagenin acetate).

3) Identification of these two degradation products proves that the second hydroxyl group is located in the $C_{23}$–$C_{27}$ part of the cholegenin molecule. This hydroxyl is primary, as the chromic acid oxidation of cholegenin leads to a keto-acid, which can be reduced again to cholegenin by means of lithium aluminium anhydride.

4) The hypothesis that any steroid $C_{27}$ will have the cholesterol skeleton, together with the above-mentioned facts, leads to the spirostane formula for cholegenin. It further follows that the primary hydroxyl group must be located at $C_{26}$. *Iso*-cholegenin is also an *iso*-spirostane derivative and therefore different from cholegenin in its configuration at $C_{25}$.

The reactivity of the $C_{26}$-hydroxyl group is different in the two isomers (in *iso*-cholegenin it is considerably impeded sterically). On the strength

of this difference, which expresses itself also in the infrared spectrum, it is possible to assign to the hydroxyls at $C_{26}$ the equatorial and polar conformation in cholegenin and *iso*-cholegenin, respectively.

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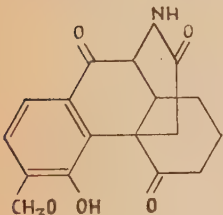
### The Synthesis of Morphine

The dioxolactam (I) has been converted to racemic dihydrothebainone (II). Resolution of the latter substance with *D*-tartaric acid permitted the isolation of *L*-dihydrothebainone which was identical with *L*-dihydrothebainone from natural sources.



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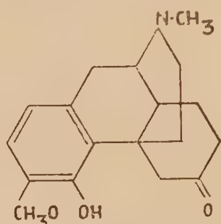

II

This synthesis constitutes the total synthesis of morphine since dihydrothebainone has been converted to morphine by previous investigators.

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### Aluminium Soaps as Gellifying Agents

An attempt has been made to establish the relationship between chemical constitution of the fatty acid and the properties of the gel produced by its aluminium soap, when dissolved in an organic non-polar liquid.

2-Ethyl-hexoic acid and derivatives, mostly substituted in the 2 position, were used.

Also aluminium soaps of fatty acids having from 5 to 10 C atoms in the chain have been synthetized and the corresponding gels tested.

The results of these experiments show that there is a definite influence of the side-chain on the properties of the gels.

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### Studies in the Alicyclic Series

The synthesis and reactions of 2-phenyl-cyclopent-2-enone will be discussed. The use of 1-benzoylcycloalkenes and 1-acetyl-cycloalkenes as acceptors in the Michael condensation will be described. It is possible by employing these reagents to obtain interesting tricyclic intermediates. The employment of the Michael condensation in the potential synthesis of tricyclic benzenoid and non-benzenoid aromatic hydrocarbons will be discussed.

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### Synthesis of 2,5-Dichlorostyrene

For some technical uses (potting materials), the polymer of 2,5-dichlorostyrene has certain desirable properties. Although this substance has been produced on a technical scale, no details on the synthesis of the monomer have been published. A practical method of preparation has, therefore, been worked out.

The ethylation of *p*-dichlorobenzene (I) with gaseous ethylene in presence of aluminium chloride yields 2,5-dichloroethylbenzene (II) in 35—40% yield (b.p. 80.5 °/8 mm; $d = 1.239$). The reaction is accompanied by partial dechlorination of the starting material, and in 25—30% yield, *p*-chloro-ethylbenzene is formed (b.p. 48.5 °/8 mm; 169 °/760 mm). Its structure was proven by oxidation to *p*-chlorobenzoic acid with boiling concentrated nitric acid. Furthermore, a higher-boiling fraction, consisting of higher-ethylated *p*-dichlorobenzenes was observed.

Bromination of (II) with bromine or *N*-bromosuccinimide yields a monobromoderivative in 95% yield; b. p. 141.5 °/5 mm, $d^{16} = 1.5800$; $n_D^{21.5} = 1.5880$. It is likely that this compound has formula (III), although the infrared spectrum did not show the band characteristic of the methyl group.

Dehydrobromination with alcoholic sodium hydroxide solution gives in excellent yield the desired 2,5-dichlorostyrene (IV), b.p. 92-94 °/5 mm, $d^{20} = 1.2460$, $n_D^{20}$; $= 1.5298$. The compound polymeriszes easily under standard conditions.

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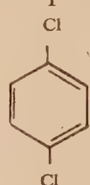

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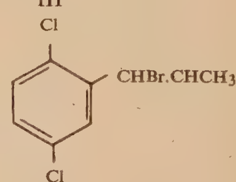

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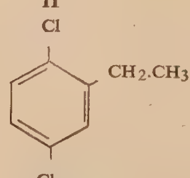

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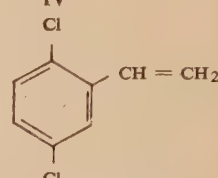

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### New Syntheses of 9,10-Diphenyl-phenanthrene and 3,4-Benzphenanthrene

1) The construction of the phenanthrene system by the interaction of phenyl-lithium with compounds of the 2,2′-bis-(bromomethyl)-biphenyl type, opens a new route into the series of 3,4-benzphenanthrene. 1-(*o*-Bromomethyl-phenyl)-2-bromomethyl-naphthalene was prepared and converted, by reaction with phenyl-lithium, into 9,10-dihydro-3,4-benzphenanthrene. Dehydrogenation with palladium gave 3,4-benzphenanthrene.

2) Wittig and Witt[1] have shown that the reaction of diphenylbromomethane with phenyl-lithium yields 1,1,2,2-tetraphenylethane. Therefore, the reaction of phenyl lithium with 2,2′-bis-(α-bromobenzyl)-biphenyl was studied, in order to see whether this secondary bromide is also capable of *intra*-molecular cyclisation. Indeed, 9,10-diphenyl-9,10-dihydro-phenanthrene was obtained; it could be dehydrogenated to 9,10-diphenylphenanthrene.

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### Reversible Colour Changes in Spiropyrans and Merocyanins at Low Temperatures

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### A New Synthesis of Thio-orotic Acid

When a mixture of thiourea and diethyl oxaloacetate is subjected to azeotropic distillation with toluene in presence of toluene-*p*-sulphonic acid as catalyst, and the product hydrolysed and acidified, thio-orotic acid is obtained in good yield. It was identified with an authentic sample by mixed melting point and the ultraviolet spectrum.

The mechanism of the reaction and its application to other derivatives of thiouracil will be discussed.

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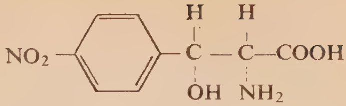
### Studies in the Chloromycetin Series

1) The direct condensation of aromatic aldehydes with glycine esters in alcoholic solution leads to the esters of the corresponding β-aryl-serines. In the case of *p*-nitrobenzaldehyde, it has been shown that with an excess of the aldehyde the *erythro*-form (I) is obtained[1,2], whilst with an excess of the amino-ester the *threo*-form (II) is the only product of the reaction; corresponds in its configuration to the antibiotic

I



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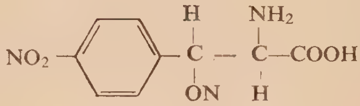

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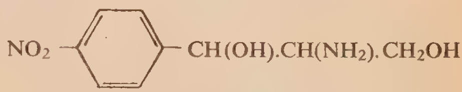

substance chloromycetin[2,3]. When the condensation is carried out by azeotropic distillation of the components with benzene, the *erythro*-form is obtained[2].

It seemed, therefore, surprising that in Erlenmeyer's classical synthesis of phenylserine by alkaline condensation of benzaldehyde and glycine only the *threo*-compound should be formed. Indeed, a closer study showed that the temperature at which the reaction is carried out has a profound influence on its steric course. This is shown in Table I[4,5].

TABLE I
*Relative yields (%) of the isomeric phenylserines*

| *Temp.* | —10° | —5° | 0° | +5° | +10° | +15° | +20° | +25° | +30° |
|---|---|---|---|---|---|---|---|---|---|
| Crude phenyl-serine | 0 | 11 | 28 | 74 | 76 | 81 | 76 | 77 | 90 |
| Ester hyd-drochlorides (total)* | — | 63 | 60 | 77 | 82 | 84 | 72 | 83 | 82 |
| *threo*-salt* | — | 2 | 18 | 55 | 54 | 69 | 67 | 78 | 82 |
| *erythro*-salt* | — | 62 | 42 | 22 | 29 | 15 | 4 | 5 | 0 |

* Calc. on crude phenylserine.

A more extensive study of the behaviour of various aromatic aldehydes showed that in most cases the different methods of condensation give the *erythro*-form. The particular situation prevailing in the case of *p*-nitro-benzaldehyde is paralleled only by 6-nitro-3-fluorobenzaldehyde, *p*-cyanobenzaldehyde and *p*-methylsulfonyl-benzaldehyde, i.e. by the aldehydes which carry in *p*-position strongly *meta*-directing substituents. One would, therefore, expect that also from *p*-carbethoxy-benzaldehyde both diastereomeric phenyl-serines would be available. These aldehydes also show the highest reactivity in such condensation as the Perkin reaction[6], but the connection between the two phenomena is yet unclear. It is worthy of note that also quinoline-4-

aldehyde and 8-nitroquinoline-4-aldehyde condense smoothly with glycine ethyl ester; the configuration of the products formed has not been established so far.

2) In an attempt to clarify the differences between the diastereomeric forms, the condensation of carbonyl compounds with the esters of the two phenylserines and the two (*p*-nitrophenyl)-2-amino-1,3-diols (III) was studied. No significant difference in rate of reaction could be detected — which would have indicated different conformation of the diastereomers, and the configuration was maintained in each case. Thus, the following substances were isolated:

*N*-Cyclohexylidene-*threo*-phenylserine ethyl ester, m.p. 61—62° (from ether-methanol)
*N*-Cyclohexylidene-*erythro*-phenylserine ethyl ester, m.p. 64—65° (from ether-methanol)
*threo*-5-(*p*-Nitrophenyl)-2-dichloromethyl-4-hydroxymethyl-oxazolidine, m.p. 175—176° (from methanol)
*erythro*- ,m.p. 203—204° (from methanol)
*threo*-5-(*p*-Nitrophenyl)-2,2-diethyl-4-hydroxymethyl-oxazolidine, m.p. 124—125° (from hexahydrotoluene)
*erythro*- ,m.p. 131—132° (from acetone-ligroin)
*threo*-5-(*p*-Nitrophenyl)--2-pentamethyleno-4-hydroxymethyl-oxazolidine, m.p. 107—108° (from acetone-ligroin)
*erythro*-- ,m.p. 125—126° (from acetone-ligroin)

Whilst the aminodiol gives in all condensations oxazolidines, the infrared spectrum proved the Schiff base structure for the products from the phenylserine ethyl esters and cyclohexanone. This is the more surprising as cyclohexanone has so far given in such cases exclusively oxazolidine derivatives[7]. From the infrared spectrum one could also infer that the secondary and not the primary hydroxyl group participates in the formation of the oxazolidine ring; the vicinity of the phenyl group appears to increase its reactivity.

3) If in (II) or its esters the carboxyl group could be reduced conveniently without affecting the aromatic nitro-group, the chloromycetin base (III, *threo*) would be available from *p*-nitrobenzaldehyde and glycine esters by an extremely simple, two-step process. Such a method has now been found in the reduction of the ethyl ester of (II) with sodium borohydride and lithium chloride in tetrahydrofuran as solvent[8]. Both diastereomers of (III) could thus be obtained in satisfactory yield.

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## ω-Fluoroacetophenone

Acetophenone is metabolized partially to benzoic acid which appears in the urine as hippuric acid. A possible mechanism for this conversion involves carboxylation to benzoylacetic acid and subsequent cleavage to benzoic and acetic acid. Therefore it could be assumed that by analogy ω-fluoro-acetophenone (I) would serve *in vivo* as a source of fluoroacetic acid. A toxicological study of this ketone was thus indicated, We report here on the synthesis and reactions of (I).

Friedel-Crafts condensation of benzene and fluoroacetyl chloride, when carried out in ethylene dichloride or methylene chloride gives up to 80% yield of I. This ketone reacts with the usual carbonyl reagents without involvement of the fluorine atom. The result of Grignardization with phenylmagnesium bromide depends on the temperature and reaction period: when the mixture is refluxed in ether and then left overnight, desoxybenzoine is the sole product. If however the reaction is carried out at 0° and limited to 1/2 hour, 1,1-diphenyl-2-fluoro-ethanol (II) of m.p. 70° is obtained in 66% yield (*Anal.* Calcd. for $C_{14}H_{13}OF$: C, 77.8; H, 6.0%. Found: C, 77.8; H, 6.2%). It appears that desoxybenzoine results from an intramolecular rearrangement of (II).

Although dehydration of (II) with boiling 25% sulphuric acid was not successful, the carbinol underwent condensation with maleic anhydride to form the fully aromatized naphthalene derivative III. This demonstrates the complete analogy between 1,1-diphenylvinyl fluoride, chloride and bromide in the Wagner-Jauregg reaction.

Upon condensation with benzaldehyde the fluorinated benzalacetophenone (IV) is formed, m.p. 60° (*Anal.* Calcd. for $C_{15}H_{11}OF$: C, 79.6; H, 4.9%. Found: C, 79.6; H, 5.1%).

Reduction of (I) with lithium aluminium hydride at 0° gave an 80% yield of 1-phenyl-2-fluoro-ethanol (V) of b.p. 55—59° (2 mm). This oily product was characterized by its *p*-nitrobenzoate of m.p. 90° (*Anal.* Calcd. for $C_{15}H_{12}O_4NF$: C, 62.3; H, 4.2%. Found: C, 62.3; H, 4.6%).

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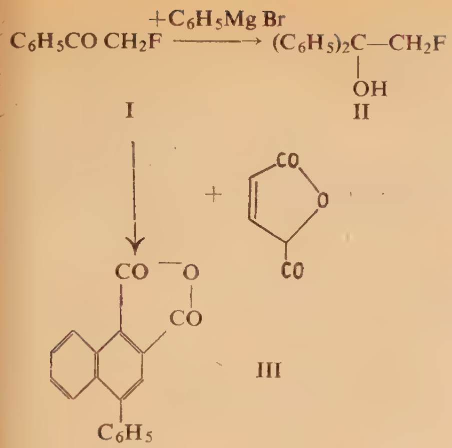

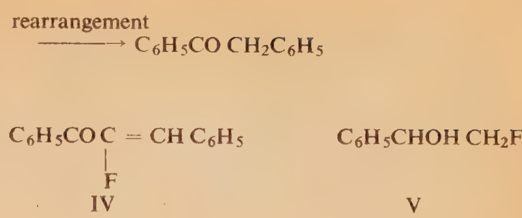

## New Reactions of Ethyl Fluoroacetate

It is possible to carry out the typical reactions of a reactive methylene group with ethyl fluoroacetate.

According to Huisgen[1], the activation of the methylene group by various activating substituents is additive. Whilst it is not possible to alkylate ethyl *acetate* in the same manner as diethyl malonate, it has been found that ethyl *fluoroacetate* behaves like diethyl malonate and exhibits a reactive methylene group. It appears that the strong inductive effect of the fluorine atom supports the carbethoxy group sufficiently so as to eliminate one hydrogen atom of the methylene group as proton.

Following these speculations, the following compounds have been prepared from ethyl fluoroacetate: a) ethyl α-fluoro-β-phenyl propionate, b) diethyl monofluorosuccinate, c) ethyl α-fluoro-α-allyl-acetate.

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## Synthesis of Fluorine-substituted Aromatic Amines

Contrary to the usual synthesis of fluoro-aromatic compounds, the decomposition of nitro-aryl diazonium fluoroborates often leads to explosions, and the yields of the nitro-fluoro-derivatives and, therefore, of the amines, available from them by reduction, are uncertain. Following some old observations[1], the diazotisation of mono-acetylated diamines was studied. The 1,3- and 1,4-diamino-derivatives give smoothly diazonium fluoroborates which can be decomposed to the fluorinated acetyl-amines. The monoacetyl derivatives of 1,2-diamines lead, upon diazotisation, to heterocyclic compounds, but their *N*-phthaloyl derivatives can be subjected to the above sequence of reactions. As the monoacyl derivatives of the diamines can be prepared either directly from the latter or by acylation of the corresponding nitroamines and subsequent catalytic reduction, the method can be satisfactorily applied in every case. It can be summarised in the following formula:

Table I gives the overall yield in a number of cases studied.

TABLE I

*Synthesis of fluorine-substituted aromatic amines*

| *Starting material* | *Final product* | *Overall yield (%)* |
|---|---|---|
| *N*-Acetyl-*p*-phenylenediamine [a] | *p*-Fluoroacetanilide | 82 |
| *N*-Acetyl-*m*-phenylenediamine [b] | *m*-Fluoroacetanilide | 75 |
| *N*-Acetyl-benzidine [c] | 4-Fluoro-4'-aminobiphenyl | 50 |
| *N*-Acetyl-*o*-dianisidine [d] | 4-Fluoro-4'-acetamino-2,2'-dimethoxy-biphenyl | 37 |
| 2-Acetamido-4'-aminobiphenyl [e] | 2-Acetamido-4-fluoro-biphenyl | 59 |
| *N*-Acetyl-naphthylene-1,4-diamine [f] | 1-Acetamido-4-fluoro-naphthalene | 69 |
| *N*-Acetyl-naphthylene-1,5-diamine [g] | 1-Acetamido-5-fluoro-naphthalene | 55 |
| $N^1$-Phthaloyl-1-2-phenylene-diamine [h] | 2-Fluoro-aniline | 47 |
| $N^1$-Phthaloyl-4-methoxy-1,2-phenylenediamine [i] | Phthaloyl-4-methoxy-2-fluoroaniline | 36 |
| $N^4$, $N^{4'}$-diphthaloyl-3,3'-diaminobenzidine [j] | Diphthaloyl-3,3'-difluoro-benzidine | 25 |

Yield from
a) *p*-nitroacetanilide 98%
b) *m*-nitroacetanilide 96%
c) benzidine 71%
d) *o*-dianisidine 74%
e) 2-acetaminobiphenyl (2 steps) 68%

Yield from
f) 4-nitro-1-acetamido-naphthalene 93%
g) 5-nitro-1-acetamido-naphthalene 88.5%
h) *N*-phthaloyl-*o*-nitroaniline 93.5%
i) 2-nitro-4-methoxy aniline (2 steps) 68%
j) 3,3'-diamino-4,4'-di(phthalimido)-biphenyl 84.5%

In a number of cases, it was observed that, contrary to the general rule, the diazonium fluoroborates were soluble in water. Surprisingly enough, it was found that addition of copper powder at room temperature causes the desired decomposition of the diazonium salts to the fluoro-aromatic compounds in good yield. Other anions present did not cause any competitive substitution, a phenomenon which must be related to the presence of an additional —NHAc group in the molecule.

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## III. *Biochemistry*

### Enzymatic Hydrolysis of Carboxylic Acid Anhydrides

The fact that tetraethyl pyrophosphate possesses a much higher affinity for esterases than phosphoric esters indicates that these enzymes should in general split anhydrides faster than esters. However the rapid spontaneous hydrolysis of acyl anhydrides in dilute aqueous solutions prevents an experimental verification of this general rule. Therefore a new method was worked out, in which a heterogeneous organic phase continuously delivers small amounts of the substrate to the enzyme phase ("two-phase technique"). The rapid enzymatic decomposition of acyl anhydrides could be demonstrated in two reactions:

a) Hydrolysis by specific and unspecific esterases, by measuring the liberation of $CO_2$ from a bicarbonate buffer.

b) Enzymatic esterification of choline by propionic and butyric anhydride to yield propionyl- and butyrylcholine resp., which were determined colorimetrically.

Unfortunately the behaviour of a mixed carboxylic—phosphoric anhydride (dibenzyl acetyl phosphate) could not be established unequivocally, since these liquid compounds undergo spontaneous disproportionation to a pyrophosphate and a carboxylic acid anhydride.

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### Studies on the Nature of the Enzyme Hydrogenase

An enzyme exists in certain bacteria whose substrate is molecular hydrogen. This enzyme, hydrogenase, in its interaction with hydrogen, simulates active platinum. The enzyme can catalyze the reduction with hydrogen of nitrate, fumarate, methylene blue, etc., and catalyze the exchange reaction:

$$HD + H_2O \rightleftharpoons H_2 + HDO \qquad (1)$$

as well as the conversion of para-to ortho-hydrogen. Previous investigations suggest that this enzyme contains ferrous iron.

Comparative studies of the rates of the exchange reaction between deuterium gas and water and of the ortho-para conversion indicate that both recations involve the intermediate formation of an enzyme hydride as shown below:

$$H_2 + E \rightleftharpoons H : E^- + H^+ \qquad (2)$$

in which $E$ represents the iron containing enzyme. The effect of certain inhibitors (CO, $HCN^-$, NO, etc.) will be discussed in connection with a hypothesis on the evolutionary development of the iron containing pigments of the living cell.

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### The Action of Water-soluble Synthetic Polyamino Acids on the Clotting of Fibrinogen

Purified bovine fibrinogen was clotted with partially purified bovine thrombin in isotonic barbital buffer solutions, pH 7.3, ionic strength 0.15.

Clotting times were plotted against thrombin concentrations (Figure 1) and against fibrinogen concentrations (Figure 2).

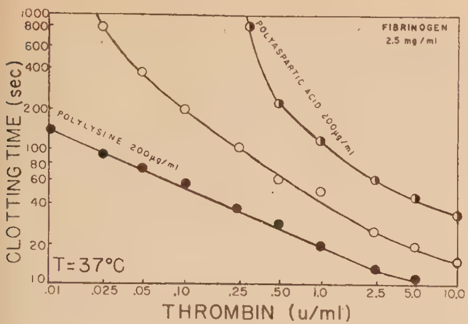


Figure 1

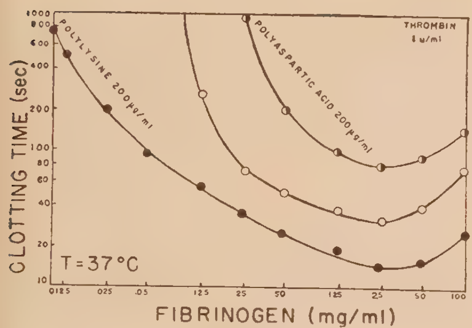


Figure 2

The basic polyamino acids poly-*L*-lysine hydrobromide, poly-*DL*-arginine sulphate and poly-*DL*-ornithine hydrochloride of average chain length 30—60 amino acid residues per molecule markedly shortened the clotting times when added to the clotting mixtures.

The acidic polyamino acids poly-*L*-aspartic, poly-*L*-glutamic and poly-*L*-cysteic acids of average chain length of 80, 49 and 10 amino acid residues per molecule, respectively, prolonged the thrombin fibrinogen clotting times.

The neutral polyamino acid poly-*DL*-alanine as well as the various amino acid monomers had no influence on the clotting times. The general shape of the inhibition and acceleration curves did not differ from that of the control.

When very diluted fibrinogen solutions were acted upon by thrombin, no clotting occurred during many hours of observation. However, in the presence of polylysine clots were produced with thrombin even in fibrinogen concentrations as low as 0.01 mg/ml (Figure 2). This action of polylysine may prove useful in the detection of minute quantities of fibrinogen. Purified fibrinogen solutions were also clotted with papain and with partially purified staphylocoagulase preparation[1]. In both cases a shortening of the clotting times occurred upon the addition of basic polyamino acids. No effect was observed in the presence of the acidic and neutral polyamino acids.

The accelerating activity of the basic polyamino acids may be a result either of their combination with thrombin which acts as a clotting catalyst, or of their combination with the substrate fibrinogen. In the former case it should be assumed that the thrombin polybase interaction increases thrombin activity, while in the latter case it should be assumed that the fibrinogen polybase complex renders the substrate more susceptible to the clotting action of thrombin.

As fibrinogen solutions become turbid in the presence of the basic polyamino acids, the formation of such a complex with fibrinogen seems probable. In this connection it is pertinent that thrombin splits off the fibrinogen molecule a polypeptide[2,3] which is acidic due to a large content of glutamic and aspartic acid residues[4,5].

Thus it is possible that the thrombin action is facilitated due to the combination of the basic polyamino acids with the acidic polypeptide, either while still attached to the fibrinogen or after its cleavage. On the other hand the clot decelerating activity of the acidic polyamino acids may be due to the increase in concentration of acidic peptide chains in the clotting milieu.

It is known that basic polyamino acids act as inhibitors of many biological processes such as fibrinolysis[6], blood thromboplastic activity[7], proteolysis by pepsin[8], clearing of alimentary lipemia by heparin[9], bacterial and virus growth[10,11]. The present observation seems to be the first to show an acceleration of an enzymatic reaction by basic polyamino acids.

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## Interaction of Basic Poly-amino Acids with Human Red Blood Cells

The interaction between negatively charged cells and positively charged (basic) poly-electrolytes is very pronounced; the poly-bases are able to agglutinate red blood cells or bacteria in concentrations as low as a fraction of a microgram per ml.

This paper deals with the nature of the combination of basic poly-amino acids, — of which synthetic poly-*DL*-lysine was chosen as representative, — with red blood cells (which were chosen for their relative simplicity and well known properties).

The investigation was carried out mainly by electrophoretic measurements, under well defined conditions of pH and ionic strength.

The electrophoretic mobility of red blood cells in poly-lysine solutions increases (positive sign) with increasing concentrations of poly-lysine, due to adsorption of the positively charged polyelectrolyte on the negatively charged surface of the cell. It has been shown that *this adsorption is a reversible reaction, reaching a state of equilibrium*; consequently, *the electrokinetic potential* of the red blood cell in poly-lysine solution *is a single valued function of the equilibrium concentration of free poly-lysine in solution.*

A method is described whereby mobility data can be used as sensitive indicators from which both the concentration of poly-lysine in solution and the amount adsorbed on the cells can be evaluated.

An adsorption isotherm of the Freundlich type has been obtained, which is characteristic of the interaction of poly-lysine with the red blood cell at a given pH and ionic strentgh.

The site of poly-lysine adsorption in the red blood cell has been shown to be in the membrane only, — the poly-lysine does not penetrate into the interior of the cell. However, the adsorption by the membrane is not all surface adsorption—the greater part is adsorbed within the membrane structure.

Comparative data for poly-lysine adsorption on the surface of glass particles and their electrophoretic mobility have been obtained. It was found that the increase in the net positive charge of the surface is not equivalent to the number of positive lysine residues adsorbed, reaching only up to 1% of the total number adsorbed.

Poly-lysine adsorption by the membrane, and its hemolytic effect are discussed in relation to red cell membrane structure.

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## The Influence of Detergents on the Respiration of Microorganisms

The effects of detergents on the respiration of *Pseudomonas fluorescens* and the spores of *Penicillium roqueforti* were investigated. Cationic detergents were bactericidal and high concentrations caused the agglutination of cells of *Ps. fluorescens*. Anionic detergents neutralized the cationic ones. This neutralization, however, could only be effected if the anionic detergent was added before or together with the cationic detergent. The effect of the cationic detergent proved irreversible if the anionic detergent was added after 15 seconds. The anionic detergents did not inhibit bacterial respiration in Warburg respiration experiments. On the contrary, they slightly increased the oxygen consumption of *Ps. fluorescens* on many substrates. Sodium taurocholate itself supported respiration to a significant degree.

Experiments with the spores of *Penicillium roqueforti* showed that anionic detergents accelerate the respiration of the spores in a Warburg apparatus. The autorespiration is negligible. The investigation is continued.

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## The Bacillomycins, a New Family of Antibiotic Polypeptides

In recent years, a series of antifungal agents synthetized by *Bacillus subtilis* have been reported. One of these, bacillomycin R, has been studied in this laboratory[1]. Further work showed it to be very similar to bacillomycin and fungocin. A chemical and biological comparison of the above three substances, as well a comparison with four other antifungal agents described in the literature, strongly suggests that all the seven belong to one family. The name "bacillomycin" is suggested for this family of antifungal antibiotics.

These seven products of *B. subtilis* are non-dialyzable, heat-stable, acid polypeptides. Their physico-chemical and biological properties are highly similar, though there are strong indications for the non-identity of these antifungal antibiotics. The characteristics causing the differences between the various bacillomycins have been studied.

Aside from the theoretical implications of this work, practical applications may be found in the treatment of fungus diseases of plants and animals.

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## Problems Connected with Fumigation of Grain with Ethylene Dibromide*

Fumigation of cereal grains with ethylene dibromide (EDB) against granary pests has been practised in Israel for several years. This measure has proved to be very efficient, but in several poultry farms the feeding of fumigated grain caused a decrease in the size of the eggs, or a complete cessation of egg production.

In order to investigate these harmful effects of EDB and to prevent them in the feeding of EDB—fumigated grain the following experiments were carried out.

1) *Determination of the amount of bromine present in the grain after fumigation.*

It was found that bromine was present in the grain in two forms after fumigation:

a) as EDB, soluble in chloroform. After fumigation the grain contains large quantities of EDB (up to 500 p.p.m.) but this evaporates within a period of approximately 6 weeks, when no chloroform soluble bromine is recovered from the grain.

b) as material insoluble in chloroform. This bromine appears to be present in the grain as the result of a reaction between EDB and constituents of cereal grains. The quantity of bromine not soluble in chloroform, found in EDB fumigated grains, was very small (maximum 100 p.p.m.) but this bromine is combined in a stable fashion and does not evaporate even after prolonged aeration of the grain (6 months).

Characteristic differences were found in the amount of EDB absorbed by different cereal grains. The amount of absorbed EDB in descending order is as follows: corn, sorghum, wheat, barley.

These differences are the result of the different morphological structure of the seed coats and are not due to different chemical composition.

2) *Nutrition experiments with laying hens.*

Nutrition experiments were arranged in which laying hens received sorghum grains which contained varying quantities of EDB. The feeding of grain containing in the average 250 p.p.m. of free EDB for 6 weeks caused a cessation of egg production after this time.

The feeding of grain containing only 10 p.p.m. of bromine in the form of EDB caused after 3 months a decrease in the weight of the egg from an average of 60 gram, to an average of 50 gram.

The feeding of grain which did not contain free EDB but contained large quantities of bound bromine (as much as 120 p.p.m.) had entirely no effect on the egg production.

Suitable aeration, which ensures the freeing of the grains from EDB, prevents any harmful effect from feeding fumigated grain.

3) *The mechanism of action of EDB*

We presume that the insecticide action of EDB and its harmful effects on fowls are caused by its reaction with —SH groups. We therefore examined the effect of EDB on 2 enzymes which contain the —SH radical as an active group, namely succinic oxidase and urease. It was found that the EDB vapour inhibited the activity of solutions of these enzymes almost entirely after one hour incubation at 37 °C.

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* This investigation forms part of a thesis submitted by Eugenia Olomucki to The Hebrew University of Jerusalem in partial fulfilment of the requirements for the degree of Ph.D.

## Paper-chromatographic Separation of Substituted Uric Acids

For studies on the metabolism and excretion of xanthines a method is required for the determination of methylated uric acids in the presence of large amounts of uric acid in serum and urine. So far the only method available involves destruction of uric acid by uricase, which does not attack the higher homologs. However, enzymatic degradation is never complete and thus requires complicated extraction methods.

We have succeeded in paper-chromatographic separation of uric, monomethyl- and dimethyluric acids, which permits the determination of 5 gamma of the latter in the presence of large amounts of the former. Since alkyl groups increase the solubility in the organic phase, it is to be expected that the substituted uric acids migrate faster than the mother substance. Thus a mixture of butanol 70, formic acid 15 and water 15% by volume gives the following $R_F$ values.

| | |
|---|---|
| Uric acid | 0.06 |
| 3- methyluric acid | 0.22 |
| 1,3-dimethyluric acid | 0.57 |

The greatest difficulty is the detection of the spots, since the usual molybdate reagent reacts with all reducing substances present and even with the paper itself. Whe have observed that the mercuric-complexes of uric acids show up as violet spots under ultraviolet irradiation. Since however, the mercuric-complexes of alkyl-uric acids

are water soluble, it is necessary to spray the paper with an organic solution, in which all these complexes are precipitated on the paper. The suitable reagent is a dioxane solution of mercuric acetate.

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## On the Reversible Inhibition of Pepsin by Polylysine

In a previous communication[1] we have shown that the enzymatic activity of pepsin is inhibited by polylysine. The inhibition is reversible and resembles the inhibition of pepsin by its natural inhibitor[2]. The concentration of the pepsin-polylysine complex as a function of pepsin is given in Figure 1 for three different concentrations of polylysine.

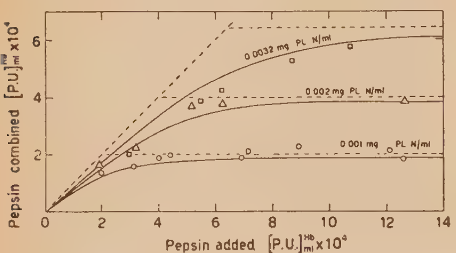


Figure 1

The effect of increasing amounts of pepsin and poly-*L*-lysine (PL) (average degree of polymerization $n=36$) on the amount of pepsin bound by the inhibitor at pH 1.7. The points are the determined values. The solid lines are the theoretical curves calculated from the mass law as indicated in the text. The broken lines indicate the course of the reaction if it were stoichiometric, i.e., $K=0$.

The activity of the pepsin was 0.10 $[P.U]^{Hb}_{mgN}$ and the average degree of polymerisation of the polylysine was 36. The maximum amount of pepsin which combined 4.6μ polylysine (0.001 mg PL N) was 3.6μg ($2 \times 10^{-4}$ $[P.U]^{Hb}$). Since the molecular weight of pepsin is 38,000 and that of the polylysine used 4,600, the pepsin-polylysine complex contains 2.7 molecules of polylysine per each molecule of pepsin.

In the following the equilibrium between the inhibitor and the enzyme is considered, assuming each molecule of the enzyme to combine with one molecule of the inhibitor. In such a case the law of mass action gives:

$$\frac{(\text{Pepsin})\ (\text{Inhibitor})}{(\text{Pepsin — inhibitor})} = K$$

If we denote by:

$P_t$ — the total concentration of pepsin

$P_c$ — the concentration of pepsin-polylysine complex

$I_t$ — the total concentration of the inhibitor,

the above equation becomes:

$$\frac{(P_t - P_c)\ (I_t - P_c)}{P_c} = K$$

Introducing $P_c = nI_t$, where $n$ stands for the fraction of the inhibitor which has combined with the enzyme ($0 \leqslant n \leqslant 1$), the equation takes the form

$$P_t = nI_t \quad \frac{nK}{1-n}.$$

This equation shows that the inhibitor will combine completely with the enzyme ($n=1$) only when the concentration $P_t$ of the latter equals infinity. At a high ratio of $P_t$ to $I_t$, a large increase in enzyme concentration will produce but little change in the concentration of the enzyme-inhibitor complex.

The slope of the curve $P_c$ versus $P_t$, which is given by

$$\frac{dP_c}{dP_t} = \frac{P_c - I_t}{2P_c - P_t - I_t - K}$$

takes the form $I_t/(I_t + K)$ at the origin, where $P_c$ and $P_t$ equal zero. The initial slopes at given $I_t$ values permit the calculation of the dissociation constant $K$. If $K=0$, i.e., when the enzyme-inhibitor complex does not dissociate, the initial slope is 45°. At the other extreme when the complex dissociates completely, i.e., $K=\infty$, the initial slope is zero.

The dissociation constants of the pepsin-polylysine complex $K = 0.5 \cdot 10^{-4}$ $[P.U.]^{Hb}_{ml}$ was calculated from the initial slopes of the curves given in Figure 1. The broken lines in the Figure show $P_c$ as a function of $P_t$ for the case $K=0$.

Turbidimetric measurements of pepsin-polylysine mixtures showed that the turbidities of solutions containing varying concentrations of enzyme and peptide at the pH values 2 and 3 do not differ significantly from those containing pepsin only. At the pH values 4 and 5 and ionic strengths 0.1 and 0.2, when the concentration of pepsin was kept constant (50 μg/ml) and the concentration of polylysine was varied, the turbidity curve had a maximum at a concentration of 20μg/ml of poly-*L*-lysine hydrobromide. At the maximum turbidity the molar ratio of pepsin to polylysine is 1 to 2.

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## Poly-α-Amino Acid Derivatives of Proteins and Peptides

Becker and Stahmann[1] have recently proved that a polyglycine derivative of bovine serum albumin can be prepared by polymerizing *N*-carboxyglycine anhydride in aqueous solution in presence of the protein. The free amino groups of the protein serve as initiators of a polymerization that proceeds according to scheme I below.

A considerable part of the anhydride is, of course, hydrolysed to glycine. In a similar way Fraenkel-Conrat[2] has attached polyleucine to tobacco mosaic virus, to bovine serum albumin and to insulin.

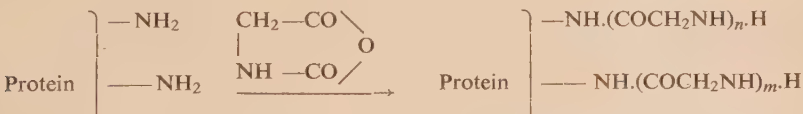
This method has been used in our laboratory to prepare polytyrosyl-gelatin, as well as branched molecules composed either of lysine and alanine residues, or of lysine residues only.

Gelatin, unlike native proteins, is not antigenic. Several authors have explained this fact by the absence, or the low content, of tyrosine in gelatin[3,4]. It was, therefore, thought of interest to attach tyrosine residues to the gelatin molecule and to study the antigenic properties of the new substance. *O*-Carbobenzoxy-*N*-carboxy-*L*-tyrosine anhydride[5] was polymerized in dioxane-aqueous solution at pH 7 (phosphate buffer and) 0° in the presence of a gelatin containing tyrosine less than 1% of the dry weight. The carbobenzoxy groups of the polycarbobenzoxytyrosyl-gelatin were removed with anhydrous hydrogen bromide[6], and the substance obtained was purified from low molecular weight by-products by prolonged dialysis. In experiments in which the anhydride was polymerized in the presence of different amounts of gelatin, tyrosyl-gelatin-polymers were obtained, which contained 7—16% tyrosine, as determined spectrophotometrically (2935 A and pH 13). If 38,700 be assumed as the average molecular weight[7] of the gelatin used, it appears that 16—48 tyrosine residues were attached to the gelatin molecule under the experimental conditions described. Unlike polytyrosine[5], which is soluble only at pH values higher than 10, the different preparations of polytyrosyl-gelatin are soluble in water, acids and bases. The antigenic properties of the gelatin derivatives prepared are being investigated.

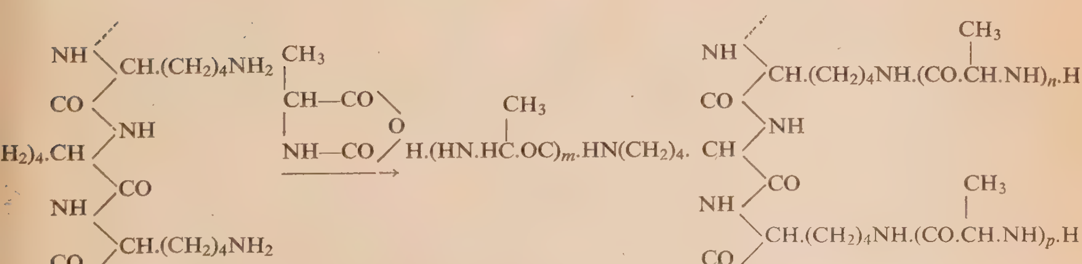
Poly-*L*-lysine[8] contains a considerable number of free ε-amino groups, and can, therefore, be used as a polyvalent initiator in the polymerization of *N*-carboxyamino-acid anhydrides. The polymerization of *N*-carboxy-*DL*-alanine anhydride in dioxane-aqueous solution at pH 7 and 0° in the presence of poly-*L*-lysine (average degree of polymerisation 30) yielded a branched polymer, in accordance with scheme II below.

Poly-*DL*-alanyl-poly-*L*-lysine was purified from the linear poly-*DL*-alanine by prolonged dialysis. The molar ratio between alanine and lysine (17:1) in the branched polymer prepared was calculated from the quantitative chromatography of the acid hydrolyzate of the copolymer. An independent support for this ratio is obtained from the determination of the terminal groups[9] of the copolymer. Taking into consideration the average length of the poly-*L*-lysine chains, it seems that the poly-*DL*-alanyl-poly-*L*-lysine obtained has a molecular weight of about 40,000. Measurements of the viscosity and diffusion coefficient of the solution support the assumption that the copolymer is of a relatively high molecular weight.

Polymerization of $N^{\varepsilon}$-carbobenzoxy-$N^{\alpha}$-carboxy-*L*-lysine anhydride in the presence of poly-*L*-lysine, under conditions similar to those given




Scheme I




Scheme II

above, yielded a branched polymer from which, after removal of its carbobenzoxy groups with anhydrous hydrogen bromide[6], branched polylysyl-polylysine was obtained.

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## The Terminal groups of Polypeptides Prepared from N-Carboxy-α-amino Acid Anhydrides

In a previous communication[1] we have shown that in the polymerization of *N*-carboxy-α-amino acid anhydrides, in addition to initiation (a) and propagation (b), a termination reaction (c) occurs:

$$\text{(a)}\ \mathrm{XH} + \mathrm{O}\begin{array}{l}\diagup \mathrm{CO{-}CHR} \\ \qquad\quad | \\ \diagdown \mathrm{CO{-}NH}\end{array} \longrightarrow \mathrm{X.CO.CHR.NH_2} + \mathrm{CO_2}$$

(I)

$$\text{(b)}\ \mathrm{X.[CO{-}CHR{-}NH]_{\mathit{n}}.H} + \text{(I)} \longrightarrow \mathrm{X.[CO{-}CHR{-}NH]_{\mathit{n}+1}.H} + \mathrm{CO_2}$$

$$\text{(c)}\ \mathrm{X.[CO{-}CHR{-}NH]_{\mathit{m}}.H} + \text{(I)} \text{ --- } \mathrm{X.[CO{-}CHR{-}NH]_{\mathit{m}}{-}CO.NH.CHR.COOH}$$

In the reaction (b) each growing chain bears an active free amino group capable of interacting with the monomer. The termination reaction (c) leads, however, to chains containing terminal carboxyl groups adjacent to urea groupings. As these carboxyl groups are incapable of reaction with the monomer, the polymerization process ceases at this stage. Consequently a polymer obtained by water-initiation contains: 1. Carboxylic groups adjacent to peptide bonds — their number being equal to the number of initiator molecules; 2. Carboxylic groups adjacent to urea groupings — in terminated chains; 3. Amino groups, formed by the propagation reaction — in unterminated chains. In amine-initiated polymers only the amino groups (3) and the carboxylic groups (2) are present.

If $A$ be the total number of carboxylic terminal groups per amino acid residue, $B$ — the total number of amino terminal groups per amino acid residue, and $C$ — the number of amino-initiator molecules, the number average degree of polymerization, $DP$, may be calculated from the following expressions:

If water-initiated $\qquad DP = 2/(A+B)$

If amine-initiated $\qquad DP = 1/(A+B) = 1/C$

If both amine- and water-initiated $\qquad DP = 2/(A+B+C)$

If $U$ be the number of carboxylic groups adjacent to urea groupings, per amino acid residue then, since each molecule has two ends, the following equation holds:

$$B + U = C + (A - U)$$

The amount of amino groups in different polymers was determined according to Van Slyke[2], or by anhydrous titration with perchloric acid, using thymol blue as an indicator[1]. The total amount of carboxylic groups was determined by anhydrous titration with sodium methoxide, using thymol blue as an indicator[1]. The amount of carboxylic groups adjacent to urea groupings is found by the colorimetric determination of the hydantoin-acetic acid derivatives obtained quantitatively on acid hydrolysis[3] according to the following scheme:

$$\mathrm{R_1{-}CO.CHR.NH.CO.NH.CHR.COOH} \xrightarrow{\text{HCl 6N}} \mathrm{R_1H} + \begin{array}{l}\mathrm{CHR{-}CO}\diagdown \\ | \qquad\qquad\quad \mathrm{N{-}CHR.COOH} \\ \mathrm{NH{-}CO}\diagup\end{array}$$

(II)

TABLE I

| Poly-α-amino Acid | Conditions of Polymerization | A | B | C | U Calc. | U Found | DP |
|---|---|---|---|---|---|---|---|
| Poly-*L*-lysine [a,b] | in benzene, 80° | 0.118 | 0.059 | — | 0.030 | 0.031 | 11 |
| Poly-*L*-lysine [a,b] | in benzene, 80° | 0.120 | 0.052 | — | 0.034 | 0.031 | 12 |
| Poly-*L*-lysine [b] | in bulk, 120° | 0.083 | 0.019 | — | 0.032 | 0.037 | 20 |
| Poly-*L*-lysine, [b,c] | in dimethyl-formamide, 20° | 0.030 | 0.020 | — | 0.030 | 0.030 | 20 |
| Poly-*L*-lysine [b,d] | in dioxane, 20° | 0.016 | 0.012 | 0.029 | 0.016 | 0.018 | 36 |
| Poly-*L*-phenylalanine [c] | in dioxane, 20° | 0 | 0.024 | — | 0 | 0.002 | 42 |
| Poly-*L*-phenylalanine [c] | in dioxane, 102° | 0.034 | 0 | — | 0.034 | 0.031 | 29 |
| Poly-*L*-phenylalanine | in bulk, 110° | 0.056 | 0.010 | — | 0.023 | 0.018 | 30 |

[a] Water-initiated. [b] *A* and *B* were determined before the decarbobenzoxylation of the polycarbobenzoxylysine. [c] Initiated by diethylamine. [d] Initiated by tyrosine ethyl ester.

The colorimetric method worked out for the quantitative determination of hydantoin-acetic acid derivatives is based on their reaction with picric acid in an alkaline solution. The amount of the coloured compound obtained is determined after acidification with acetic acid at 5000A.

The amount of the initiator was conveniently determined in some cases when tyrosine ethyl ester was used to initiate the polymerization. The number of tyrosine residues was determined spectrophotometrically at 2935A and pH 13.

The values obtained for the terminal groups of several poly-α-amino acids prepared under different conditions are given in Table I.

The Table shows that amine-initiated polymers contain carboxylic groups adjacent to urea groupings, thus confirming the occurrence of the termination reaction (c). Moreover, it appears that the termination reaction is temperature-dependent, and that at higher temperatures mainly terminated chains were formed, while at lower temperatures most of the chains remained unterminated.

It ought to be mentioned that the number average molecular weight of poly-α-amino acids, containing a significant proportion of terminated chains, is considerably lower than the value calculated from the amount of amino-terminal groups only, considered hitherto to be the correct one.

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## The Kinetics of the Polymerization of N-Carboxy-α-amino Acid Anhydrides

Sela and Berger[1] have shown recently that the polymerization of *N*-carboxy-α-amino acid anhydrides proceeds as follows:

$$\text{Initiation: } Cat.\mathrm{H}+m_o \rightarrow Cat.m_1(\mathrm{NH_2})$$

$$\text{Propagation: } Cat.m_j(\mathrm{NH_2})+m_o \xrightarrow{k_1} Cat.m_{j+1}(\mathrm{NH_2})$$

$$\text{Termination: } Cat.m_j(\mathrm{NH_2})+m_o \xrightarrow{k_2} Cat.m_{j+1}(\mathrm{COOH})$$

where *Cat*.H denotes the initiator containing an active hydrogen, and $m_o$ the anhydride. $m_j$ represents a polymeric chain of *j* amino acid residues.

The kinetics of polymerization may, therefore, be described by the series of equations given below, where $N^*_j$ denotes the concentration of *growing* chains of degree of polymerization *j*, containing free amino terminal groups, while $N_j$ denotes the concentration of the corresponding *terminated* chains, containing terminal carboxyl groups. *L* and (*Cat*) represent the concentrations of the anhydride $m_o$ and the catalyst, respectively, at time *t*. $L_o$ and $(Cat)_o$ are the concentrations at time $t = 0$. $k_1$ is the propagation constant and $k_2$ the termination constant. It is assumed that the rate of reaction of the catalyst (usually an amine) with the monomer is determined by $k_1$. $\varkappa$ denotes $k_1+k_2$.

$$-d(Cat)/dt = \varkappa\,(Cat)L$$

$$d\overset{*}{N}_j/dt = k_1\overset{*}{N}_{j-1}L - \varkappa\,\overset{*}{N}_jL$$

$$dN_j/dt = k_2\overset{*}{N}_{j-1}\cdot L$$

Introducing the new variable $\nu$ defined by

$$\nu = \int_0^t L\,dt \qquad d\nu = L\,dt$$

we obtain as the solution of the above equations:

$$(Cat) = (Cat)_o\,e^{-\varkappa\nu}$$

$$N_j^* = (Cat)_o\,[(k_1\nu)^j/j!]\,e^{-\varkappa\nu}$$

$$N_j = (Cat)_o\,[k_2k_1^{j-1}/(j-1)!]\int_0^\nu \nu^{j-1}e^{-\varkappa\nu}\,d\nu$$

Figure 1 illustrates $N^*_j$ and $N_j$ as a function of the degree of polymerization *j* at different time values $\varkappa\nu$, when $k_1$=0.10 and $k_2$=0.003. The figure shows that the distribution of the growing chains approaches a Poisson distribution. No maximum appears in the distribution of the terminated chains.

The ratio of the numbers of terminated to unterminated molecules given by:

$$(\mathrm{COOH})/(\mathrm{NH_2}) = \sum_{j=1}^{\infty} N_j / \sum_{j=o}^{\infty} N^*_j) = e^{k_2 \nu} - 1$$

shows that the number of terminated chains increases with $\nu$, i.e. with time, while the member of growing chains decreases correspondingly.

The weight distribution of both terminated and unterminated chains for the case illustrated in Figure 1 is given in Figure 2.

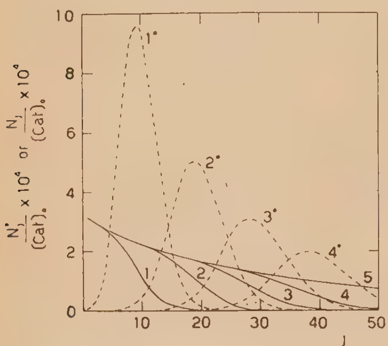


Figure 1

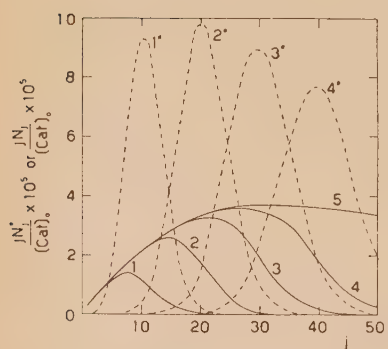


Figure 2

When $k_1 \gg k_2$ and $k_1 \gg 1$, the number and weight average molecular weights, $\bar{M}_n$ and $\bar{M}_w$ respectively, of the terminated and unterminated fractions are given by:

$$\bar{M}_n^* \approx M_o \,.\, k_1 \nu \qquad \bar{M}_n \approx M_o \,.\, (k_1/k_2)$$

$$\bar{M}_w^* \approx M_o \,.\, k_1 \nu \qquad \bar{M}_w \approx 2M_o \,.\, (k_1/k_2)$$

where $M_o$ is the m.wt. of the amino acid residue. For the fraction composed of growing chains $\bar{M}^*_n \approx \bar{M}_w^*$, while for the fraction of terminated chains $\bar{M}_w \approx 2\bar{M}_n$. The former fraction resembles ethylene oxide polymers in this respect, while the latter resembles polycondensates and vinyl polymers.

Preliminary experiments on the kinetics of polymerization and on the composition of different polyamino acids, carried out in this laboratory, support the above considerations. The ratio $k_2/k_1$ was found to decrease with decreasing temperature. Therefore, polymers of a higher degree of polymerization can be obtained at lower temperatures.

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### Decarbobenzoxylation by Lithium Bromide

Ben-Ishai and Berger have shown that the carbobenzoxy groups of *N*-carbobenzoxyamino acids and *N*-carbobenzoxy-peptides may be removed by treatment with hydrogen bromide or hydrogen chloride in anhydrous organic solvents[1]. These results encouraged us to study the decarbobenzoxylation by inorganic halides. Most of the experiments were carried out with lithium bromide, which is readily soluble in a number of organic solvents. Clark and Todd have reported the use of lithium bromide in the debenzylation of phosphate derivatives[2].

On heating *N*-carbobenzoxyphenylalanine in a saturated solution of lithium bromide in pyridine to 155° during 45 minutes, phenylalanine was obtained in a 97% yield. A similar yield was obtained after 18 hours at 106°. Decarbobenzoxylation by lithium bromide also occurs in dimethylformamide and glacial acetic acid. Quantitative yields of the corresponding amino acids were also obtained from *N*-carbobenzoxyglycine and *N*-carbobenzoxyalanine.

The carbobenzoxy groups of polycarbobenzoxy-*L*-lysine and polycarbobenzoxy-*DL*-ornithine were quantitatively removed by lithium bromide in pyridine in 60 hours at 100° and in 3 hours at 160°. The peptide bonds of polyamino acids seem to be stable under these conditions, since a similar treatment of polytyrosine does not result in an increase in amino groups.

Kinetic experiments in which the amount of phenylalanine was determined by amino nitrogen analysis showed that the reaction between lithium bromide and *N*-carbobenzoxyphenylalanine is of the second order. The rate constant at 140° is $4.4 \cdot 10^{-6}$ mol litre$^{-1}$ sec$^{-1}$.

Decarbobenzoxylation of *N*-carbobenzoxyphenylalanine may also be effected by other inorganic halides. Experiments were carried out in pyridine with various salts at 160°—165°, an excess of salts being present in each case. The initial concentration of *N*-carbobenzoxyphenylalanine was 0.813 mol/litre in all cases. The yields given in the following table were determined after two hours.

TABLE

| *Salt* | *Yield in %* |
|---|---|
| LiBr | 97.0 |
| $MgBr_2$ | 87.6 |
| LiCl | 25.0 |
| $MgCl_2$ | 8.3 |
| $CH_3COOLi$ | 2.0 |
| $CH_3COOK$ | 2.0 |
| Li $NO_3$ | 0.0 |

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## Poly-L-proline

A polyamide containing proline residues exclusively is a useful model for the study of the chemical, physical and biological properties of proteins, such as collagen, gelatin and zein, which contain a high percentage of proline and hydroxyproline. Unsuccessful attempts to synthetize polyproline (III) and *N*-carboxyproline anhydride (II), have been reported[1,2].

Poly-*L*-proline has been successfully synthetized in this laboratory. *N*-Carbobenzoxy-*L*-proline (I) yielded on treatment with phosphorus pentachloride *N*-carboxy-*L*-proline anhydride (II), which was purified by molecular distillation. Poly-*L*-proline (III) was obtained from (II) by polymerization in bulk at 130°—135°, or in solution.

The structure of (III) was ascertained by combustion analysis and by the quantitative recovery of *L*-proline from its acid hydrolyzate.

Polyproline, like polysarcosine, is soluble in water, probably owing to the absence of amide hydrogens, which form intermolecular hydrogen bridges between the CONH groups in water-insoluble polyamides. Like gelatin, it forms elastic films, dissolves in water and glacial acetic acid, and may be precipitated from its aqueous solution by trichloroacetic acid. It shows the remarkably high optical rotation of $[\alpha]_D^{20} = -370^\circ$, in glacial acetic acid.

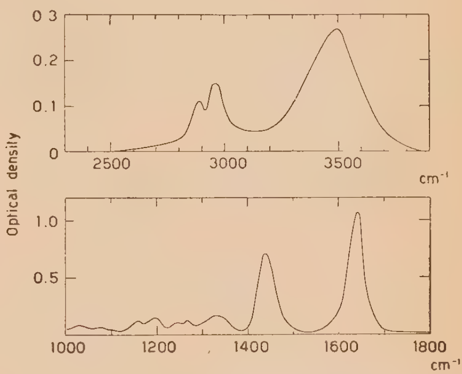
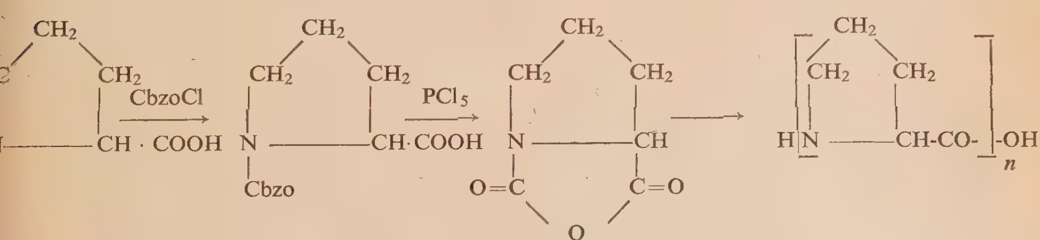



Figure 1

The infrared absorption spectrum of (III) in the range of 3800 to 1000 cm$^{-1}$ is given in Figure 1. The band at 1640 cm$^{-1}$ is presumably due to the carbonyl group, though its frequency is somewhat lower even than that of a hydrogen bounded amide. The absorption at 1438 cm$^{-1}$, 2885 cm$^{-1}$ and 2955 cm$^{-1}$ may be assigned to the CH frequencies of the proline residues. As expected, no absorption due to an NH bending frequency at 1510—1530 cm$^{-1}$ was found in the spectrum. The band at 3495 cm$^{-1}$ is not easily assigned. Polyproline does not contain OH and NH groups which absorb in this range, and since the proline residues retain their full optical activity, the formation of OH bonds as a result of tautomerization may be excluded. This absorption band may be a harmonic of the carbonyl frequency, similar to that of *N,N*-dibutyl-trifluoroacetamide[3] at 3472 cm$^{-1}$.




I (II) (III)

A Debye-Scherrer diagram of (III) showed a number of characteristic reflections corresponding to spacings of 8.5 A and 4.5 A (strong reflections) and 2.44 A, 2.01 A and 1.04 A (weak reflections). The first two spacings are present in collagen. The typical 2.86 A spacing of collagen is, however, absent in the synthetic polymer.

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## Electrophoresis of Poly-lysine, Poly-aspartic Acid, and co-polymers of Lysine-Aspartic Acid

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## Formation of Lysine Oligopeptides in the Tryptic Hydrolysis of Tri-L-lysine

It has been shown[1] that poly-*L*-lysine is readily hydrolysed by trypsin. From amino nitrogen (Van Slyke) and ninhydrin —$CO_2$ determinations it was concluded that the final digest contains mainly lysyl-lysine and a small quantity of lysine. These findings were recently confirmed by Waley and Watson[2], who followed the course of the enzymatic hydrolysis by means of paper chromatography. This technique permitted the separation and quantitative determination of the various lysine oligopeptides. In order to elucidate the mechanism of the enzymatic hydrolysis of poly-*L*-lysine, it was decided to investigate chromatographically the action of trypsin on lower lysine peptides and their derivatives.

The quantitative chromatographic analysis of the final tryptic hydrolysate of tri-*L*-lysine showed that the tripeptide is converted into lysyl-lysine and lysine. The molar concentration of the lysine dimer considerably exceeded that of the lysine monomer. Hence the dimer could not have been formed from the trimer by a simple cleavage of one of its peptide bonds. When the progress of the tryptic digestion was followed chromatographically (cf. Table), it was found that the molar concentration of the dimer exceeded that of the monomer at all stages of the reaction and that tetra-, penta- and hexa-*L*-lysine peptides appeared in small amounts in the reaction mixture after a few minutes of incubation. These oligopeptides remained in the reaction mixture at an almost constant concentration for several hours and then disappeared. Since the tetra-, penta- and hexa-*L*-lysine peptides undergo tryptic hydrolysis faster than trilysine[2], one has to assume that they are formed and hydrolysed in the course of the enzy matic hydrolysis of tri-*L*-lysine. The presence o the intermediates permits alternative routes to th formation of lysyl-lysine, which do not necessaril involve the formation of lysine. A possible rout is given below:

$$2\ \text{Ly-Ly-Ly} \rightarrow \text{Ly-Ly} + \text{Ly-Ly-Ly-Ly}$$
$$\text{Ly-Ly-Ly-Ly} \rightarrow 2\ (\text{Ly-Ly})$$

The addition of *L*-lysine to the enzymatic re action mixture did not enhance lysine peptid formation. The peptide bond is known to b endergonic and therefore it seems that the inter mediate oligopeptides mentioned were forme by a transpeptidation reaction. It thus follow that trypsin acts both as a hydrolase and as transpeptidase. The transpeptidation rate con stant seems to be considerably higher than th hydrolytic constant. as the transpeptidation re action takes place to a significant extent even a a very low concentration of reactants.

The above considerations apply to the enzy matic hydrolysis of poly-*L*-lysine by trypsin an may explain the findings concerning the final pro ducts obtained.

Formation of peptides with a greater numbe of lysine residues than that present in the origina substrate was also observed when *L*-lysine amide or *L*-lysyl-*L*-lysine as well as some of its deriva tives, were incubated with trypsin in a high con centration. The action of trypsin on *L*-lysin amide yielded dilysine, while the action of trypsi on lysyl-lysine led to the formation of trilysin and tetralysine, in addition to the many product of the degradation reaction, i.e., lysine, lysine amid lysyllysine and ammonia.

The significant difference in the rate of trypti hydrolysis of benzoyl-*L*-lysine amide and poly-*L* lysine on the one hand, and that of di-*L*-lysin and tri-*L*-lysine on the other, indicates that th terminal amino and carboxyl groups of the pep tides retard the hydrolysis of the adjacent peptid bonds. In this connection it is pertinent to not that the rate of tryptic hydrolysis of lysine oligo peptides increases with their chain length.

HYDROLYSIS OF TRI-L-LYSINE BY TRYPSIN

*The enzymatic hydrolysis was carried out in phospha buffer pH* 7.6 *at* 40 °.

*Concentration of tri-L-lysine hydrochloride* 10.23 *mg/r* (18.7 *μM/ml*).

*Concentration of trypsin* 0.26 *mg/ml.*

*The data are expressed in moles of lysine residue* × 10[8].

| Time (min.) | 0 | 15 | 45 | 100 | 180 | 360 | 1440 |
|---|---|---|---|---|---|---|---|
| Trilysine | 46.2 | 44.0 | 40.0 | 37.8 | 35.8 | 29.7 | 18.4 |
| Dilysine | 0.0 | 1.6 | 3.9 | 8.2 | 10.0 | 17.1 | 28.8 |
| Lysine | 0.0 | 0.0 | 0.0 | 0.5 | 1.0 | 1.6 | 2.2 |
| Trilysine hydrolysed | — | 2.2 | 4.0 | 8.4 | 10.5 | 16.5 | 27.9 |

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## The Structure of the Active Group of Serum Cholinesterase

Serum or pseudo-cholinesterase differs from the true enzyme characteristically by its S-shaped pS-activity curve. Its physiological role and specificity is not understood, especially since Adams and Whittaker[1] came to the conclusicn that the active surface of the serum enzyme does not contain a negative charge to attract a cationic substrate.

We have demonstrated the presence of an anionic site in the pseudo-cholinesterase and the similarity of both cholinesterases in all essential features by the following experiments:

a) Quaternary ammonium salts inhibit the hydrolysis of both cationic and uncharged esters. The inhibitory effect increases with increasing length of the *N*-substituents.

b) Glycine esters are good inhibitors.

c) The inhibition by prostigmine and eserine depends on pH changes in the same way, as was observed with the "true" enzyme.

Since both cholinesterases thus resemble each other in all essential properties, it is concluded that they fulfil the same role in conduction. However the turnover number of the pseudo-enzyme is about 100 times smaller. Therefore the efficiency of the buffer system

cholinesterase — choline ester

in the conductive process will be much lower. A study of the distribution of either enzyme in the body indicates indeed that pseudo-cholinesterase is essentially associated with tissues of low speed of conduction.

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## CORRIGENDA

Vol. III No. 1—2, p. 63, Table III and Figure 12:
The initial drop diameters $d_o$ were computed on assuming $y_i = 1$. A more reasonable assumption would be: $y_i - p_i/p = 0.196$, $p_i$ being the vapour pressure of water at 60 °C. The corresponding $d_o$ values may be obtained by multiplying in Table III by a factor of 0.42.

Vol. III No. 4, p. 437 *in* The Spectrum of Yb II, l. 10: *for ss* coupling *read JJ* coupling.
p. 448, 1. 9: *delete* At higher concentrations.

# SUMMARIES Vol. IV No. 1

FODOR, P. J.

*Studies on the Activity and Inhibition of Yeast Esterase*, Bull. Res. Counc. of Israel, 1954, 4, 1—5.
The esterases of commercial bakers' yeast (*Saccharomyses Cerevisiae* Hansen) have been investigated. Ester hydrolysis was found to have two optima, one in the alkaline range at pH 7.4, and one in the acid range at pH 2.8. While hydrolysis at the former optimum is destroyed by heating the yeast homogenate to 50°C, hydrolysis at pH 2.8 is reduced very little at this temperature and is destroyed only at 60°C. The range of specificity of this yeast esterase is rather narrow. Ethylbutyrate and its isomer butylacetate were observed to undergo hydrolysis with the highest initial velocity, reaching a maximum of 27 and 23 per cent, respectively, after 90 minutes of incubation, while ethyl caproate possessing the same initial velocity of hydrolysis is hydrolyzed maximally to 13 per cent only. For all the other esters the initial velocities of hydrolysis were lower and correspondingly also the extent of their maximum hydrolysis. Both insoluble monohydric alcohol and glycerol esters which fail to undergo hydrolysis by the yeast esterase inhibit strongly its activity toward the hydrolyzable esters. Enzymatically resistant water-soluble esters, however, do not exert any inhibitory effect. In addition also sodiumcholeate was observed to inhibit hydrolysis, although on a smaller scale.

BIRNBAUM, S. M. and GREENSTEIN, J. P.

*Peptidases in Renal and Hepatic Tissues*, Bull. Res. Counc. of Israel, 1954, 4, 6—11.
In contrast to the activity of the soluble acylase fraction of kidney with an [apparently single and inseparable specificity toward chloroacetyl-*L*-alanine, glycyl-*L*-alanine, chloroacetyldehydroalanine and glycyldehydroalanine, the comparable soluble fraction of liver, by means of differential heat inactivation experiments, has been shown to possess readily separable activities toward the three substrates, chloroacetyl-*L*-alanine, chloroacetyldehydroalanine and glycyldehydroalanine. The fact that each of these three substrates is acted upon by a separate enzyme clarifies the previous observations from this Laboratory which showed that the neoplastic transformation of liver was accompanied by a loss of the activity toward chloroacetyldehydroalanine (dehydropeptidase II), an increase in the activity toward glycyldehydroalanine (dehydropeptidase I) and no change in activity toward chloroacetyl-*L*-alanine. Again in contrast to kidney, in which the greater part of the activity toward glycyldehydroalanine, glycyldehydronorvaline and glycyldehydrophenylalanine is associated with a single activity in the particulate fraction, liver maintains the greater part of its activity toward the first substrate in the soluble fraction, shares the activity toward the second substrate between soluble and particulate fractions and maintains all of the activity toward the third substrate in the particulate fraction. In the hepatoma which develops from this liver, the soluble activities of the former two substrates increase as the corresponding particulate activities decrease, while the particulate and sole activity of the third substrate disappears.

NEUBERG, C.

*Bioreduction of Trimethylamine Oxide*, Bull. Res. Counc. of Israel, 1954. 4, 12—14.
A suspension of *Thermobacterium mobile* was mixed with a solution of glucose and incubated at 28°. When, after the addition of some magnesium fructose-6-phosphate, trimethylamine oxide was added to the fermenting mixture it was reduced to trimethylamine. This bioreduction was not accompanied by the formation of a volatile acid.

CRANE, J. and SCHOENFELD-REINER, REBECCA.

*Note on the Ageing of Bitumen*, Bull. Res. Counc. of Israel, 1954, 4, 15—17.

CHASHIN, Z. and REINER, M.

*Note on the Elasticity of Solid-Solid Dispersions*, Bull. Res. Counc. of Israel, 4, 18.

BERGMANN, E. D., SICHER, S. and VOLCANI, B. E.

*The Action of Chloromycetin, Aureomycin and Terramycin on the Biosynthesis of Indole in* Escherichia coli, Bull. Res. Counc. of Israel, 1954, 4, 19—30.

1. The inhibition of a wild strain of *Escherichia coli* by chloromycetin, aureomycin or terramycin is reversed within limits by indole and tryptophan, much less by phenylalanine and tyrosine, and not at all by anthranilic acid.
2. The analogous inhibitions of the indoleless, the tryptophanless, the phenylalanineless, the tyrosineless and the anthranilic acid-less mutants of *Esch. coli* are reversed in various degrees by the corresponding metabolites.
3. The results suggest that chloromycetin interferes with the conversion of anthranilic acid to indole. The combination of anthranilic acid, methionine and vitamin $B_{12}$ overcomes the interference. Methionine appears, therefore, to be the source of the $C_1$ moiety required for the completion of the indole skeleton.
4. The similarity in the mode of action of the three antibiotics is related to the known phenomenon of cross-resistance existing between them.
5. 5-Fluoro-tryptophan has the same mode of action as the three antibiotics, and a higher antibacterial potency.

BODENHEIMER, F. S.

*On Spontaneous Malignant Growth in the Levant Vole* (*Microtus guentheri* A.D.), Bull. Res. Counc. of Israel, 1954, 4, 31—32.

Prolonged breeding of wild voles (*Microtus guentheri* D.A.) showed that malignant tumours appear to be rather common as soon as the voles approached the age of about 2 years. Jn nature, there is little opportunity to meet animals with malignant growths because the ecological life expectancy is only about 68 days.

PATAI, S. and FREITAG, N.

*Catalytic Effect of Inorganic Salts in the Heterogeneous Oxidation of Alicyclic Compounds by Potassium Perchlorate*, Bull. Res. Counc. of Israel, 1954, 4, 33—35.

The catalytic action of various salts on the aromatization of tetralin by potassium perchlorate has been studied. The action of ferrous, ferric, vanadium, cerium and titanium salts is explained by their ability to serve as electron-carriers in an analogous manner to certain biological oxidation-reduction systems. Under the same reaction conditions, in sealed tubes, typical oxygen-carriers are inactive, while under reduced pressure, the reaction does not take place even in the presence of the most active catalysts.

FRANKEL, MAX, LIWSCHITZ, Y. and ZILKHA, A.

*Preparation of some Polymeric Peptides*, Bull. Res. Counc. of Israel, 1954, 4, 36—40.

Poly-*dl*-phenylalanine, poly-*dl*-valine and poly-glycyl-*dl*-alanine were synthesized by thermal condensation of the acylchloride hydrochlorides of the respective amino acids and dipeptides.

The formation of poly-β-alanine esters from free β-alanine methyl and ethyl esters was investigated and polymers with an average chain length of 14 units obtained.

WERTHEIMER, E. and BENTOR, VICTORIA.

*A Rapidly Developing and Rapidly Waning Effect of Triiodothyronine on the Carbohydrate Metabolism of the Rat Diaphragm*, Bull. Res. Counc. of Israel, 1954, 4, 41—43.

Intravenously administered triiodothyronine has an effect about three times as great as thyroxine on the carbohydrate metabolism of rat diaphragm. Contrary to the known effect on basal metabolism, it begins very soon and disappears after a few hours.

Diiodothyronine, dibromothyronine and thyronine are almost or completely ineffective. With regard to time, this effect of triiodothyronine appears no sooner than the effect of thyroxine.

Triiodothyronine added in vitro to the rat diaphragm medium has no stronger effect than thyroxine on the carbohydrate metabolism of the diaphragm.

MONSELISE, JOSEPH J. and EPHRAIM, ABRAHAM.

*Pectin from Sunflower Heads*, Bull. Res. Counc. of Israel, 1954, 4, 44—47.

Seed-free sunflower heads are a comparatively rich raw material for pectin production, as they contain 20—25 % thereof on dry basis. Optimal extraction conditions were studied in relation to pH, temperature and time, and 100 g of raw material were found to yield 8.9 g pectin 190°.

PICARD, L.

*Structural Pattern of Palestine* (*Israel and Cis-Jordan*), Bull. Res. Counc. of Israel, 1954, 4, 48—50.

GINSBURG, I., DE VRIES, A. and SHAFRIR, E.

*Studies on the Action of Polylysine on the Fibrinolytic Reaction*, Bull. Res. Counc. of Israel, 1954, 4, 51—56,
Polylysine inhibits dissolution of fibrin clots by plasma fibrinolysin.
Polylysine promotes clotting of partially lysed fibrinogen by thrombin.

BONDI, A. and BIRK, YEHUDITH.

*Studies on the Action of Papain on Protein Feeds*, Bull. Res. Counc. of Israel, 1954, 4, 57—62,
A study was made of the papainic digestion, under different conditions, of four different feeds (2 of plant and 2 of animal origin). The results obtained indicate that:
1) Virtually no free amino acids were liberated either when the enzyme was or was not previously activated by HCN.
2) A greater quantity of terminal groups was liberated by non-activated papain at pH 5.0 than at pH 7.5.
3) The amount of terminal groups liberated was increased by preliminary activation of papain at pH 5.0 than at pH 7.5.
3) The amount of terminal groups liberated was increased by preliminary activation of papain by HCN; this effect was more striking in experiments carried out at pH 7.5 than at pH 5.0.
4) The activation at pH 5.0 caused the liberation of more peptides of the same average chain length than in the hydrolysate prepared at the same pH with non-activated papain. Activation at pH 7.5 caused an additional breakdown of the peptides to peptides of shorter chain length.
5) A protein fraction characteristic of plant proteins, but not present in animal proteins, can be isolated from papain digests of plant proteins on addition of TCA, as reported for the corresponding pancreatic digests.

PILNIK, W. and ROTHSCHILD, GERDA.

*Pectolytic Enzymes in Tomatoes*, Bull. Res. Counc. of Israel, 1954, 4, 63—65.
Tomato pectase, free from pectinase, occurs only in the clear serum. Both enzymes are readily inhibited by surfactants. Tomato pectinase is inhibited by alkali like fungal pectinase.

KUK-MEIRI, S., SHULOV., A. and LICHTENSTEIN, N.

*Proteases of the Eggs of the Desert Locust* (Schistocerca Gregaria *Forskal*), Bull. Res. Counc. of Israel· 1954, 4, 66—68.
The proteolytic activity of glycerol extracts of developing eggs of the Desert Locust, *Schistocerca gregaria* Forskal, towards casein and leucylglycylglycine has been investigated. A distinct cleavage of casein was obtained with extracts of 8 days old or older eggs. Leucylglycylglycine was not attacked by extracts from 1—2 days old eggs, whereas extracts prepared from 5 days old or older eggs hydrolyzed this peptide strongly.

BOBTELSKY, M. and GRAUS BERTHA

*Lead Tartrate, Basic Complexes, their Behaviour, Composition and Structure*, Bull. Res. Counc. of Israel‘ 1954, 4, 69—74.
1. The composition and behaviour of lead tartrate compounds which exist in neutral and alkaline solutions were studied by the heterometric, potentiometric and conductometric methods.
2. In aqueous solutions of high alkalinity (pH$>$9.5) lead forms quantitatively with tartrate the anion complex $[Pb_4Ta''_3]^{4-}$.
3. In the pH region of $\sim$9.5 to $\sim$8.0 lead is precipitated as $[Pb_2Ta'']^0$. Between pH's$\sim$8 to$\sim$5 the latter is transformed into the insoluble PbTa which then dissolves between pH's$\sim$5 to$\sim$4.
4. The character of the compounds obtained was discussed and structural formulas were postulated.

LEWIN, M., SHILOH, M. and ALEXANDER, E.

*Measurements of "Effective Crimp Diameter"*, Bull Res. Counc. of Israel, 1954, 4, 75—77.
A method is described for estimating the degree of crimp in wool fibres. "Effective crimp diameter" is defined as corresponding in some way to "effective voltage".

Data of measurements of the "Effective crimp diameter", its reduction under various loads and its recovery are presented, showing a definite hysteresis of the crimp under these conditions.

HEIMANN-HOLLANDER, EVA and LICHTENSTEIN, N.

*Specificity of* D-*amino Acid Oxidase*, Bull. Res. Counc. of Israel, 1954, 4, 78—80.
The action of pig kidney *D*-amino acid oxidase on several *N*-alkyl derivatives of *DL*-leucine, *DL*-valine and *DL*-phenylalanine as well as on *DL*-leucinamide was investigated. Whereas these compounds are practically not attacked by *D*-amino acid oxidase, some of them, including leucinamide, markedly inhibit the action of this enzyme on amino acids.